DESIGN, SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL THIADIAZOLE (SCHIFF'S BASE) DERIVATIVES AS ANTI-TUBERCULAR AGENTS AGAINST *GLUTAMINE SYNTHETASE I*

A Dissertation submitted to

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MASTER OF PHARMACY

IN

PHARMACEUTICAL CHEMISTRY

Submitted by

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CERTIFICATE

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EXAMINERS

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“Gratitude makes sense of our past, brings peace for today and creates a vision for tomorrow”

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CONTENTS

LIST OF ABBREVIATIONS

TB	Tubercle Bacillus
WHO	World Health Organization
HIV	Human Immuno Deficiency Syndrome
MDR-TB	Multi Drug Resistant TB
XRD-TB	Extensively Drug Resistant-TB
TDR- TB	Totally Drug Resistant - TB
AIDS	Acquired Immuno Deficiency Syndrome
FDA	Food and Drug Administration
DOTS	Directly Observed Treatment Short-Course
BCG	Bacilli Calmette Guerin
μm	Micro meter
μg/ml	Micro gram/ milli litre
MTB	*Mycobacterium tuberculosis*
DNA	Deoxyribo Nucleic Acid
GS	*Glutamine synthetase*
ATP	Adenosine Tri Phosphate
ADP	Adenosine Di Phosphate
ADME	Absorption, Distribution, Metabolism, Excretion
CADD	Computer Aided Drug Design
QSAR	Quantitative Structural Activity Relationship
QSPR	Quantitative Structure–Property Relationship
HTS	High Throughput screening
SAR	Structural Activity Relationship
MABA	Microplate Alamar Blue Assay
OSIRIS	Optical, Spectroscopic and Infrared Remote Imaging System

PSA	Polar Surface Area
TPSA	Topological Polar Surface Area
LBDD	Ligand Based Drug Design
SBDD	Structure Based Drug Design
TLC	Thin Layer Chromatography
LC-MS	Liquid Chromatography Coupled with Mass Spectrometry
NMR	Nuclear Magnetic Resonance Spectroscopy
PDB	Protein Data Bank
ADT	AutoDock Tools
Kcal	Kilo Calories
µl	Micro litre
HPLC	High Performance Liquid Chromatography
MIC	Minimal Inhibitory Concentration
OECD	Organization for Economic Co-operation and Development
R_f	Retardation Factor
BACTEC	Bactenecin
Log P	Partition Co-efficient
DMEM	Dulbecco's Modified Eagle Medium

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INTRODUCTION

INTRODUCTION

TUBERCULOSIS:

Tuberculosis is a chronic granulomatous disease which is a major health problem in developing countries. About one third of the world's population is infected with *Mycobacterium tuberculosis*. As per WHO, 9 million people suffer from TB and 1.7 million die annually because of TB. In India, every year nearly 2 million people develop this disease and about 0.5 million die and is a leading cause of death from an infectious disease worldwide, after the human immunodeficiency virus (HIV)[1]. Co- infection with the HIV fuels the global TB crisis, and successful TB treatment is further complicated and hampered by the existence of multidrug- resistant (MDR) TB and extensively drug resistant (XDR) TB[2].

Fig. 1: *Mycobacterium tuberculosis*[3]

An estimated 13.7 million chronic cases were active globally, while in 2013, an estimated 9 million new cases occurred. In 2013 there were between 1.3 and 1.5 million associated deaths, most of which occurred in developing countries. The total number of tuberculosis cases has been decreasing since 2006, and new cases have decreased since 2002. Many people in the developing world contract tuberculosis because of a poor immune system, largely due to high rates of HIV infection and the corresponding development of AIDS[4].

HISTORY:

Mycobacterium originated more than 150 million years ago. The German microbiologist Robert Koch in 1882 conformed the rod shaped organism caused TB. The discovery of the Bacillus-Calmette Guerin (BCG) vaccine in 1908 and anti-tuberculosis drugs starting in 1943, offered hope for the eradication of this disease[5].

Mortality rates decreased significantly from the early to mid-20th century. The Directly Observed Treatment Short-Course (DOTS) program was introduced in 1993. In 1998 the DOTS-plus program was introduced to address multidrug resistant (MDR) TB[6].

The Food and Drug Administration (FDA), on 28 December 2012, granted accelerated approval to SIRTURO™ (bedaquiline) Tablets as a part of combination therapy in adults with multidrug- resistant TB (MDR-TB). It is the first new anti-TB drug to be approved after 1998 (rifapentine was approved in 1998) and the first anti-TB drug with a novel mechanism of action to be approved after 40 years (rifampicin was approved in 1974). It is also the first to be introduced specifically for the treatment of MDR-TB in combination with other drugs[7].

TYPES OF TUBERCULOSIS:

Tuberculosis is a contagious disease; it affects almost all the important organs of the body and are generally considered to be,

1.Pulmonary tuberculosis, 2.Extra pulmonary tuberculosis

PULMONARY TUBERCULOSIS[8] :

Pulmonary tuberculosis (TB) is when *M. tuberculosis* primarily attacks the lungs. However, it can spread from there to other organs. Pulmonary TB is curable with an early diagnosis and antibiotic treatment.

Pulmonary TB, also known as consumption, spread widely as an epidemic during the 18th and 19th centuries in North America and Europe. After streptomycin, an antibiotic, was discovered in 1944, people were able to treat and control the spread of TB. Since then, TB has been in decline. It's at its lowest recorded rate since 1953.

The upper lung lobes are more frequently affected by tuberculosis than the lower ones. The reason for this difference is not entirely clear. It may be due either to better air flow, or to poor lymph drainage within the upper lungs.

EXTRAPULMONARY TUBERCULOSIS[9]:

Tuberculosis (TB) outside the lung usually results from hematogenous dissemination. Sometimes infection directly extends from an adjacent organ. These are Miliary TB, Genitourinary TB, TB meningitis, TB peritonitis, TB pericarditis, TB lymphadenitis, TB of bones and joints, Gastrointestinal TB, TB of the liver and other sites. Symptoms vary by site but generally include fever, malaise, and weight loss. Diagnosis is most often by sputum smear and culture and, increasingly, by rapid molecular-based diagnostic tests. Treatment is with multiple antimicrobial drugs given for at least 6 months.

TREATMENT [10,11]:

TB can usually be cured. More than twenty drugs have been developed for treating TB. But most of the drugs were developed many years ago. The treatment usually consists of a combination of TB drugs that must be taken for at least six months. But the treatment will only be successful if the drugs are taken exactly as required for the entire length of time. The drugs are used in different combinations in different circumstances. For example, the five "first line" drugs are given to people who have never had treatment before.

Some of the drugs have very severe side effects and are very difficult to take for such a long period of time. This is why there is an urgent need for new TB drugs to be developed. In addition many people are now resistant to one or more of the drugs.

DRUG RESISTANT TB[12]:

If someone has drug resistant TB it means that the bacteria in their body won't be affected by certain drugs that they are resistant to. The drugs just won't work. There are two main reasons why people develop it. It can be because the person doesn't take their drugs properly. It can also be that the bacteria that they are infected with, have come from someone who has already got drug resistant TB. Being drug sensitive is the opposite of being drug resistant.

If someone has drug resistant TB then they must change drugs. But usually they mustn't have just one new drug. They need to have several new drugs and for it to be believed that they will all be effective. Drug susceptibility testing which is available in many countries, and is very important, provides information about which drugs a person is resistant to.

Multi Drug-Resistant Tuberculosis (MDR TB) is caused by organism resistant to both Isoniazid and Rifampicin which are the most effective anti Tb drug[13].

Extensively Drug-Resistant Tuberculosis (XDR TB) is a relatively rare type of drug resistant TB, which is resistant to both Isoniazid and Rifampicin, plus any other Fluoroquinolone and at least one of three injectable second line[14].

Totally Drug–Resistant Tuberculosis (TDR-TB) in which the TB strains that showed *in-vitro* resistance to all first and second line drugs tested (isoniazid, rifampicin, streptomycin, ethambutol, pyrazinamide, ethionamide, para-aminosalicylic acid, cycloserine, ofloxacin, amikacin, ciprofloxacin, capreomycin, kanamycin)[15].

MYCOBACTERIA[16]:

Mycobacterium tuberculosis has an unusual, waxy coating on its cell surface (primarily due to the presence of mycolic acid), which makes the cell impervious to gram staining. *Mycobacterium tuberculosis* is the rod-shaped, spore forming aerobic bacterium.

Mycobacterium tuberculosis and seven very closely related mycobacterium species (*M. bovis, M. africanum, M. microti, M. caprae, M. pinnipedii, M. canetti and M. mungi*) are together known as *M. tuberculosis* complex.

SCIENTIFIC CLASSIFICATION[16]:

Kingdom	:	Bacteria.
Phylum	:	Actinobacteria.
Class	:	Actinobacteria.
Order	:	Actinomycetales.
Suborder	:	Corynebacterineae.
Family	:	Mycobacteriaceae.
Genus	:	Mycobacterium.
Species	:	*Mycobacterium tuberculosis*.
Synonym	:	Tubercle bacillus Koch 1882.

CELL WALL STRUCTURE[17,18]:

The cell wall is composed of two segments, upper and lower. Beyond the membrane is peptidoglycan (PG) in covalent attachment to arabinogalactan (AG), which in turn is attached to the mycolic acids with their long meromycolate and short alpha-chains. This is termed the cell wall core-the mycolyl arabinogalactan–peptidoglycan (mAGP) complex. The upper segment is composed of free lipids, some with longer fatty acids complementing the shorter alpha-chains, and some with shorter fatty acids complementing the longer chains.

Interspersed within are the cell-wall proteins, the phosphatidyl Inositol Mannosides (PIMs), the phthiocerol containing lipids, lipomannan (LM), and lipoarabinomannan (LAM). When cell walls are disrupted, for instance extracted with various solvents, the free lipids, proteins, LAM, and PIMs are solubilized, and the

mycolic acid–arabinogalactan–peptidoglycan complex remains as the insoluble residue. In simplistic terms, it can be considered that these lipids, proteins, and lipoglycans are the signaling, effector molecules in the disease process, whereas the insoluble core is essential for the viability of the cell and should be addressed in the context of new drug development.

The importance of understanding the biosynthesis of the mycolic acid–peptidoglycan–arabinogalactan complex is more in the context of new drug development against tuberculosis (TB) and less in defining the bacterial factors responsible for the disease process.

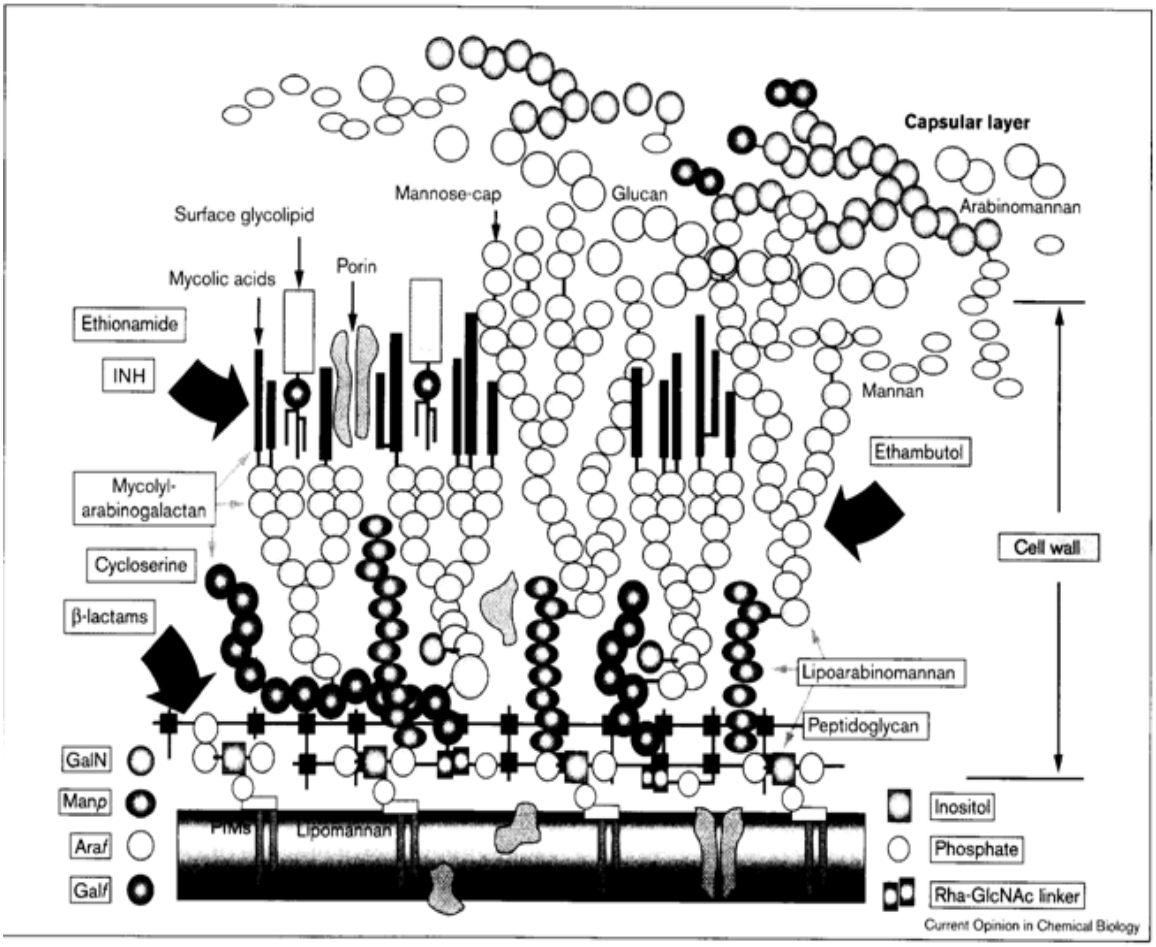
This peptidoglycan, which forms the backbone of the mAGP, consists of alternating units of N-acetylglucosamine and N-glycolylmuramic acid.

The arabinogalactan polymer is comprised exclusively of D-galactofuranoses and D-arabinofuranoses which are extremely rare in nature. The polysaccharide is also unusual in that, unlike many bacterial polysaccharides, it is composed not of repeating units but a few distinct, defined structural motifs.

Mycolic acids are large, alpha-alkyl branched, and beta-hydroxylated fatty acids. Two families of mycolic acids are known: a mycolates without any oxygenated functional groups and the oxygenated mycolates.

Slow growing pathogenic mycobacteria such as M. tuberculosis modify their acids by cyclopropanation, whereas rapid growing saprophyte species such as *M. smegmatis* do not. The mycolic acids also occur within the fluid matrix in the form of free trehalose dimycolate (TDM) (‘cord factor’) and trehalose monomycolate (TMM); both of these components are distributed on the cell surface. TMM is regarded as a precursor of the arabinan-linked mycolates, and TDM has been implicated in the pathogenesis of tuberculosis.

The other dominant feature of the mycobacterial cell wall is the LAM which is somewhat embedded into the framework of the mAGP.



A model of the mycobacterial cell wall. The three-dimensional and spatial arrangement of the key molecules are largely unknown. It is thought that most mycobacterial cell walls conform to this model, whereby mAGP and LAM are the two principal constituents. A capsular-like substance surrounds the bacillus and is this rich in polysaccharides. These extracellular polysaccharides are usually glycogen (glucan), arabinomannan and mannan [5]. The surface glycolipids include a variety of species- and strain-specific glycopeptidolipids, lipooligosaccharides, and phenolic glycolipid, the chemical identity and amount of which varies from one species to another. The sites of action of some known antimycobacterial drugs are also depicted (green arrow) in the model.

Fig. 2: Cell wall structure of *Mycobacterium tuberculosis*[17]

GENOME:

Mycobacterium tuberculosis encodes about 190 transcriptional regulators: 13 σ factors, 11 two-component systems, 5 unpaired response regulators, 11 protein kinases, and more than 140 other putative transcriptional regulators. Several of these regulators have been characterized; some of them respond to environmental stresses such as cold shock, heat shock, hypoxia, iron starvation, surface stress, and oxidative stress, while others respond to still unknown environmental conditions[19].

Mycobacterium tuberculosis has circular chromosomes of about 4,200,000 long nucleotide. The genome was studied generally using the strain Mycobacterium tuberculosis H37Rv. The genome has about 4000 genes. Genes that code for lipid metabolism are very important part of the bacterial genome and 8% of the genome is involved in its activity[19].

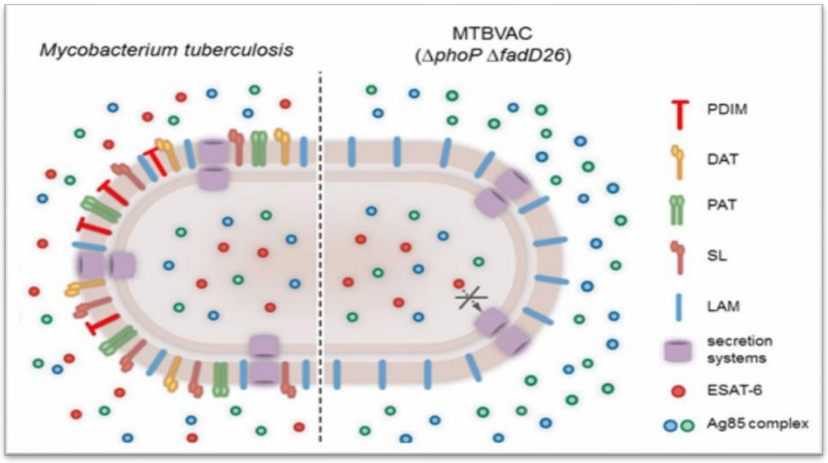


Fig. 3: Genome of *Mycobacterium tuberculosis*[20]

MODE OF TRANSMISSION[21]:

TB is usually spread through cough, sneeze, speak, sing, or spit, they expel infectious aerosol droplets 0.5 to 5.0µm in diameter. A single sneeze can release up to 40,000 droplets. They transmit the disease, since the infectious dose of tuberculosis is small.

The probability of transmission from one person to another depends upon the several factors including duration of exposure, effectiveness of ventilation, the virulence of *M. tuberculosis* strain, the level of immunity of uninfected person.

If someone does become infected, it typically takes three to four weeks before the newly infected person becomes infectious enough to transmit the disease to others.

PATHOPHYSIOLOGY [22]:

Mycobacterium tuberculosis is classified as acid- fast gram- positive bacteria due to their lack of an outer cell membrane. It divides every 15-20 hours. Which is extremely slow compared to other bacteria, it is a small bacilli that can withstand weak disinfectants and can survive in a dry state for weeks.

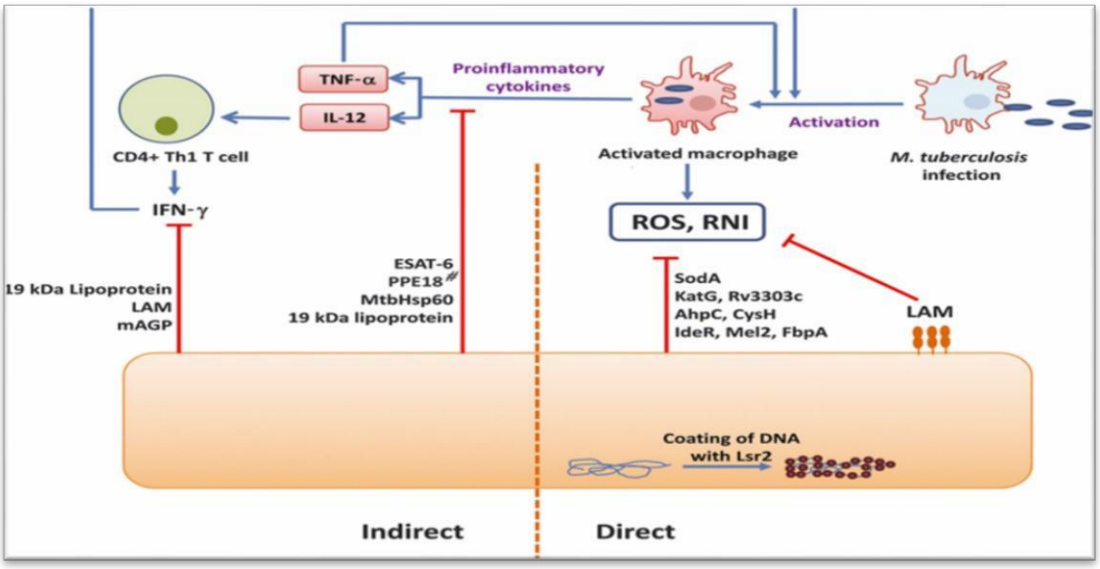


Fig. 4: Pathophysiology of Tuberculosis[23]

NEED TO FOCUS ON TUBERCULOSIS DISEASE[24]:

- Tuberculosis is a leading cause of death worldwide**.**
- In the year 2000-2005, there were around 10 to 15 million people with latent TB in the U.S & in 2007, 2.4 million cases were reported.
- In the year 2010, WHO estimated that one-third of the world's population (two billion people) was infected with the bacilli. Of the two billion people, approximately 9.8 million people will develop active tuberculosis and 2.6 million people will die per annum.
- In the year 2013, approximately 13.7 million chronic active cases were globally detected in the year 2014, approximately 560 thousand new cases per year and 740 thousand new patients infected by both MTB and HIV due to multidrug-resistant Tuberculosis.

NEED FOR NEW ANTI-TB DRUGS[25]:

- To improve the treatment of MDR-TB.
- To improve current treatment by shortening the total duration of the treatment.
- To counter the new resistant form of TB.
- To design new drugs that are more active against slowly growing and non growing persistent bacilli.
- To discover compounds that would reduce both the length of treatment and the frequency of drug administration.
- To provide more effective treatment for latent tuberculosis infection.

ENZYME PROFILE[26,27]:

GLUTAMINE SYNTHETASE I

Antibiotics typically target key cell wall or intracellular molecules of microorganisms that are involved in cell wall, protein, or DNA synthesis, or in an essential metabolic pathway. On the basis of studies in this report, we shall propose an additional site for antibiotic targeting: extracellular enzymes released by a bacterium.

M . tuberculosis , along with other pathogenic mycobacteria, is unusual among bacterial species in that it secretes or otherwise releases a large number of proteins in considerable quantities into its extracellular milieu. Such extracellular proteins are released by *M. tuberculosis* organisms when growing either in broth medium or intraphagosomally in human mononuclear phagocytes, the bacterium's primary host cells. Approximately 100 proteins are released into broth medium by *M. tuberculosis*, 11 of which are released in great abundance.

One of the abundantly released proteins is the enzyme glutamine synthetase, which is surprising because this enzyme is typically located in the bacterial cytoplasm. Even more surprising, only pathogenic mycobacteria such as *M. tuberculosis* and *M. bovis* release large amounts of glutamine synthetase extracellularly, whereas nonpathogenic mycobacteria, such as *M. smegmatis* and *M. phlei*, and nonmycobacterial microorganisms, such as *Legionella pneumophila* and *Escherichia coli*, do not.

Glutamine synthetase is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine:

Glutamate + ATP + NH3 → Glutamine + ADP + phosphate

Glutamine synthetase (GS; EC 6.3.1.2, also known as γ-glutamyl: ammonia Ligase) catalyzes the adenosine 5´-triphosphate (ATP) dependent condensation between glutamate and ammonia, to give glutamine. There are three types of GS, each with a different structure. These enzymes are present in all organisms; eukaryotes express GS type II while prokaryotes mainly express GS types I and III.

The release of *Glutamine synthetase* by pathogenic mycobacteria is correlated with the presence of a polyl -glutamate/glutamine component in the cell walls of these organisms; nonpathogenic mycobacteria lack this component . This suggests the possibility that extracellular glutamine synthetase is involved in the synthesis of polyl -glutamate/glutamine and that the enzyme's extracellular presence is significant to virulence.

In this report, we shall demonstrate that an irreversible inhibitor of *M. tuberculosis* extracellular glutamine synthetase blocks bacterial multiplication both in broth medium and in human mononuclear phagocytes and that growth inhibition is correlated with a marked reduction in the amount of the virulence-associated cell wall component polyl -glutamate/glutamine.

Remarkably, the enzyme inhibitor has no effect against nonpathogenic mycobacteria, which do not export glutamine synthetase. Although the inhibitor of glutamine synthetase may target additional extracellular proteins, our report provides strong evidence for the concept that targeting extracellular proteins of pathogenic mycobacteria and perhaps other pathogens is a feasible strategy for developing new antibiotics.

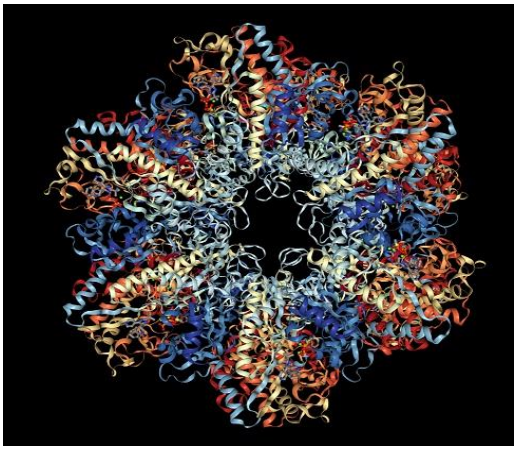


Fig. 5: *Glutamine synthetase* Enzyme having 5 chains[28]

MECHANISM[27]:

Glutamine synthetase catalyzes the ATP dependent condensation of glutamate with ammonia to yield glutamine. The hydrolysis of ATP drives the first step of a two part, concerted mechanism. ATP phosphorylates glutamate to form ADP and an acyl phosphate intermediate, γ-glutamyl phosphate, which reacts with ammonia, forming glutamine and inorganic phosphate. ADP and Pi do not dissociate until ammonia binds and glutamine is released. ATP binds first to the top of the active site, glutamate binds near the second cation binding site at the bottom of the active site. The presence of ADP causes a conformational shift in GS that stabilizes the γ-glutamyl phosphate moiety. Ammonium binds strongly to GS only if the acylphosphate intermediate is present. Ammonium, rather than ammonia, binds to GS because the binding site is polar and exposed to solvent. In the second step, deprotonation of ammonium allows ammonia to attack the intermediate from its nearby site to form glutamine. Phosphate leaves through the top of the active site, while glutamine leaves through the bottom (between two rings).

DRUG DESIGN[29]:

Drug discovery process involves a rapid search for a small molecule often called as lead. A lead molecule is chemical compound which possess pharmacological or biological activity. Sources of lead compounds can come from natural sources, such as plants, animals, or fungi and also from synthetic chemical libraries.

LEAD IDENTIFICATION/OPTIMIZATION[30,31]:

Lead identification/optimization is the one of the most important steps in drug development. The chemical structure of the lead compound is used as a starting point for chemical modifications in order to improve potency, selectivity, or pharmacokinetic parameters.

RATIONAL DRUG DESIGN[32]:

A. Development of small molecules with desired properties for targets, biomolecules (proteins or nucleic acids), whose functional roles in cellular processes and 3D structural information are known. This approach in drug design is well established and is being applied extensively by the pharmaceutical industries.
B. Development of small molecules with predefined properties for targets, whose cellular functions and their structural information may be known or unknown. Knowledge of unknown targets (genes and proteins) can be obtained by analyzing global gene expression data of samples untreated and treated with a drug using advanced computational tools.

Once a target is identified, then both approaches (A) and (B) for development of small molecules require examination of several aspects. These aspects include, but are not limited to, the evaluation of binding scores (affinity/specificity), balance between hydrophilicity/lipophilicity, absorption, distribution, metabolism, and excretion (ADME), electrophilic, nucleophilic, and radical attack (biodegradation), toxicity of the parent small molecules, and products due to biotransformation in the different phases of metabolism, quantitative structure–activity relationship (QSAR), and quantitative structure–property relationship (QSPR) respectively. Most of these aspects including design of a small molecule could be performed initially using computational tools.

COMPUTER AIDED DRUG DESIGN[33]:

The most fundamental goal in drug design is to predict whether a given molecule will bind to a target and if so how strongly. Molecular mechanics or molecular dynamics is most often used to estimate the strength of the intermolecular interaction between the small molecule and its biological target.

Drug design with the help of computers may be used at any of the following stages of drug discovery:

1. Hit identification using virtual screening (structure- or ligand-based design).
2. Hit-to-Lead optimization of affinity and selectivity (structure-based design, QSAR, etc.).
3. Lead optimization of other pharmaceutical properties while maintaining affinity.

In order to overcome the insufficient prediction of binding affinity calculated by recent scoring functions, the protein-ligand interaction and compound 3D structure information are used for analysis.

TYPES OF DRUG DESIGN:

There are two major types of drug design.

1. Ligand- Based Drug Design and
2. Structure-Based Drug Design.

LIGAND BASED DRUG DESIGN[34]:

Ligand-based drug design is one of the popular approaches for drug discovery and lead optimization. 3D structure-activity relationships (3D QSAR) and pharmacophore modeling are the most important and widely used tools in ligand-based drug design that can provide crucial insights into the nature of the interactions between drug target and ligand molecule and provide predictive models suitable for lead compound optimization.

STRUCTURE BASED DRUG DESIGN[35,36]:

Structure-based drug design is most powerful when it is a part of an entire drug lead discovery process. structure-based design can lead to the parallel synthesis of focused compound libraries. It is also important to consider that structure- based drug design directs the discovery of a drug lead, which is not a drug product but, specifically, a compound with at least micromolar affinity for a target. Binding site identification is the first step in structure based design. If the structure of the target or a sufficiently similar homolog is determined in the presence of a bound ligand, then the ligand should be observable in the structure in which case location of the binding site is trivial.

SCREENING AND DESIGN[34,35]:

The process of finding a new small molecule [ligand] against a chosen target for a particular disease usually involves high-throughput screening [HTS].

The structure – activity relationship [SAR] is to improve certain features of the lead compound,

1.Increase activity against the chosen target.

2.Reduce activity against unrelated targets.

3.Improve the drug likeness or ADME properties of molecule.

DOCKING:

Molecular docking is generally used to detect the protein-ligand orientation and interaction.

The quality of any docking results depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results.

It includes the following steps,

1. Preparation of receptor & ligand files.
2. Calculation of affinity maps by using a 3D grid around the receptor & ligand.
3. Defining the docking parameters and running the docking simulation.

ADME ANALYSIS[37]:

Optimization of the ADME (Absorption, Distribution, Metabolism, and Excretion) properties of the drug molecule is often the most difficult and challenging part of the whole drug discovery process. The ADME profile will also have a major impact on the likelihood of success of a drug. If there is any failures, as they do not undergo these properties satisfactorily this has to be ruled out earlier in drug discovery process.

Many *invitro* studies and *insilico* tools are available to evaluate the ADME properties.

PREDICTION OF ADME RELATED PROPERTIES:

Absorption:

To investigate this *insilico* models uses simple parameters like log D [diffusion coefficient] and polar surface area are the descriptors for hydrogen bonding capacity and logp (partition coefficient) values should fall under the prescribed values as per the rule of five, which determines the absorption.

Bioavailability:

Size and shape of the molecules, lipophilicity and flexibility determines the bioavailability.

Metabolism:

Various in silico approaches are exisiting in evaluating the metabolism namely QSAR and 3D QSAR.

HETEROCYCLIC CHEMISTRY:

Heterocyclic structures always are a part in the field of research and development in organic chemistry. Millions of heterocyclic structures are found to exist having special properties and biological importance. A series of thiadiazole have been synthesized using an appropriate synthetic route and characterized by elemental analysis and spectral data.

The various thiadiazole rings are

1, 2, 4-Thiadizole

1, 3, 4-Thiadizole

1, 2, 5-Thiadizole

1, 2, 3-Thiadizole

THIADIAZOLE NUCLEUS: 1, 3, 4-THIDIAZOLE:

Thiadiazole ring system contains five-membered di unsaturated ring structure having molecular structure formula $C_2H_2N_2S$ containing two carbon atoms, two hydrogen, two nitrogens and one sulphur. The ending azole designates a five membered

ring system with two or more heteroatom, one of which is Nitrogen. Thiadiazoles are associated with diverse biological activity probably by virtue of –N=C-S- functional. 1,3,4- Thiadiazole moiety contain a heterocyclic nucleus in which sulfur is present at position -1, and two nitrogen atoms at position-3 & 4.

Thiadiazole is biologically identical to that of Pyrimidine and Ox diazole and given the prevalence of pyrimidine in nature, it is not surprising that thiadiazole show significant therapeutic potential. The Sulfur atom of thiadiazole imparts improved liposolubility.

This literature review shows that the thiadiazole nuclei have various biological activities such as

- Anti-tubercular[50,51,52,53],
- Analgesic and Anti-inflammatory[44]
- Anticonvulsant[46],
- Antidepressant[48],
- Antioxidant[49],
- Antihypertensive[47],
- Anti-fungal[45].

In view of the importance of the Thiadiazole nucleus. It was decided to design nucleus based on the Thiadiazole nucleus. One hundred different molecules with the thiadiazole scaffold were drawn and docked.

Thiadiazole is a versatile moiety that exhibits a wide variety of biological activities. Thiadiazole moiety acts as “hydrogen binding domain” with “two-electron donor system”. Many drugs containing thiadiazole nucleus are available in the market such as Acetazolamide, Methazolamide, Sulfamethazole,etc.

Thiadiazole derivatives possess interesting biological activity probably conferred to them by the strong aromaticity of this ring system, which leads to great in vivo stability and generally, a lack of toxicity for higher vertebrates, including humans. When diverse functional groups that interact with biological receptors are attached to this ring, compounds possessing outstanding properties are obtained.

BIOLOGICAL EVALUATION:

Microplate Alamar Blue Assay (MABA)[74]:

Throughput in tuberculosis drug discovery was extremely limited prior to the introduction of microplate-based susceptibility assays. The 96-well Microplate Alamar Blue Assay (MABA) allows for the quantitative determination of drug susceptibility against any strain of replicating *Mycobacterium tuberculosis* to be completed within a week at minimal cost.

Cytotoxicity[75]:

Cell culture can be used to screen for toxicity both by estimation of the basal functions of the cell (i.e. those processes common to all types of cells) or by tests on specialized cell functions. General toxicity tests, aimed mainly at detection of the biological activity of test substances, can be carried out on many cell types (e.g. fibroblasts, HeLa and hepatoma cells).

A number of parameters including vital staining, cytosolic enzyme release, cell growth and cloning efficiency are used as end-points to measure toxicity. Organ-specific toxic effects are tested using specialized cells by measuring alterations in membrane and metabolism integrity and/or in specific cell functions (e.g. glycogen metabolism in primary hepatocyte cultures, beating rate in mixed myocardial cells or myocytes, and phagocytosis in macrophages).

Cytotoxicity tests using specialized cells have proved most useful when the in vivo toxicity of a chemical is already well established and where in vitro investigations using specialized cell cultures have been used to clarify the mechanisms of toxic action on the target tissue. These tests have also provided useful insight into the pathogenesis of some human diseases.

Acute Oral Toxicity[76]:

Testing for acute toxicity is the most fundamental of toxicological investigations and is routinely performed as a regulatory requirement for a number of different substance and product types in order to ensure human safety. The results of these studies are used to characterize the hazard, to assign the substance to a classification category to provide information on the likely medical treatment.

AIM

AND

OBJECTIVE

AIM AND OBJECTIVE

AIM:

To design novel molecules with potent anti-tubercular activity which are capable of inhibiting cell wall synthesis by inhibiting *Glutamine synthetase* I. The designed molecule will be synthesized, characterized and evaluated for biological activity and toxicity.

OBJECTIVE:

The compounds are designed and docked against a specific crucial target, *Glutamine synthetase* I. This enzyme is involved in the cell wall biosynthesis. The synthesized compounds are expected to act on the specific enzyme and inhibit cell wall synthesis.

PLAN OF WORK

PLAN OF WORK

- Design of *Glutamine synthetase* I inhibitors by docking studies using **AutoDock Tools 4®** software.
- Insilico Drug likeness prediction using **Molinspiration®**.
- Insilico Toxicity Assessment using **Osiris Property Explorer®**.
- Laboratory synthesis of those compounds with top Docking Scores.
- Characterization of the synthesized compounds by,
  - TLC method
  - Melting point.
  - Infrared Spectroscopy.
  - LC-Mass Spectroscopy.
  - H^1 Nuclear Magnetic Resonance Spectroscopy.
- In-vitro anti -tubercular activity of synthesized compounds (**Microplate Alamar Blue Assay**).
- Acute Oral Toxicity studies
- In-vitro cytotoxicity studies.

THE STUDY WAS CARRIED OUT BASED ON THIS FLOW CHART:

IDENTIFICATION OF LEAD COMPOUND

LEAD OPTIMIZATION

DOCKING OF THE MOLECULE TO THE TARGET PROTEIN

TOP G SCORE COMPOUNDS SELECTED

INSILICO DRUG LIKENESS AND TOXICITY RISK ASSESSMENT

SYNTHESIS OF DESINGED MOLECULE

JUSTIFICATION OF PURITY –TLC, MELTING POINT

CHARACTERIZATION –SPECTROSCOPY(IR, NMR, LC-MS)

INVITRO ANTI TUBERCULAR ACTIVITY(MABA)

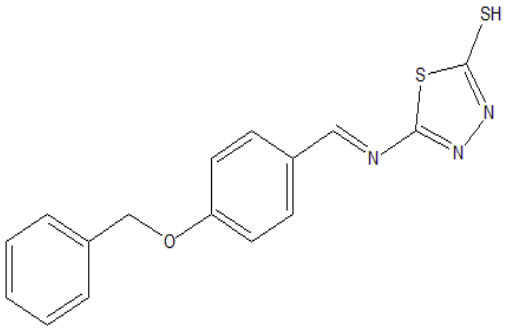
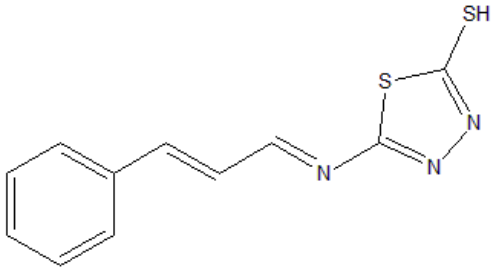
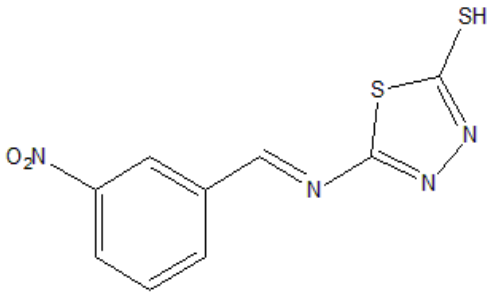
ACUTE ORAL TOXICITY STUDY

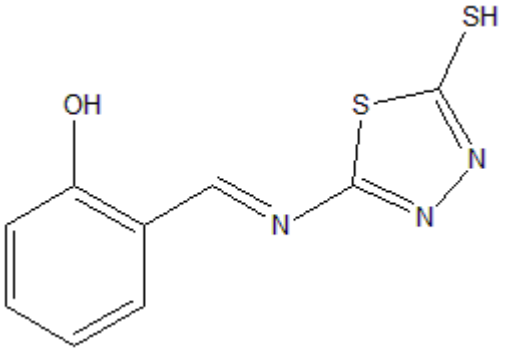
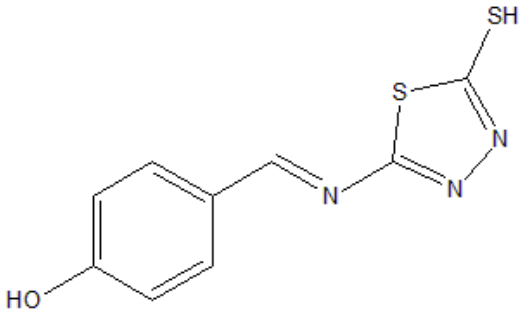
INVITRO CYTOTOXICITY STUDY

Flow chart 1: Plan of work

LIST OF COMPOUNDS TO BE SYNTHESIZED:

Table 1. List of compounds to be synthesized

SAMPLE CODE	STRUCTURE	IUPAC NAME
BOB		5 - ({(*E*) - [4-(benzyloxy)phenyl] methylidene} amino)-1,3,4-thiadiazole-2-thiol
CIN		5 - {[(1*E*, 2*E*) -3-phenylprop-2- en-1-ylidene] amino}-1,3,4-thiadiazole-2-thiol
NIB		5 - {[(*E*) - (3-nitrophenyl) methylidene] amino}-1,3,4-thiadiazole-2-thiol

SAMPLE CODE	STRUCTURE	IUPAC NAME
OHB		2 - {(*E*) - [(5-sulfanyl-1,3,4-thiadiazol-2-yl) imino] methyl}phenol
PHB		4 - {(*E*) - [(5-sulfanyl-1,3,4- thiadiazol-2-yl) imino]methyl}phenol

LITERATURE REVIEW

LITERATURE REVIEW

The purpose of Literature Review is to:

- Establish a theoretical frame work for a topic/ subject area
- Define Key terms and terminology
- Identify studies, Models, Case studies etc supporting a topic
- Define/establish an area of Study

Reviews related to the target- *Glutamine synthetase I*:

1. Gunter Harth and Marcus A. Horwitz demonstrated that inhibitors of extracellular *Glutamine synthetase* block the growth of *M. tuberculosis* and other pathogenic mycobacteria. Remarkably, the inhibitors were selective for pathogenic mycobacteria, which export *Glutamine synthetase* and contain the poly-l-glutamate/ glutamine cell wall structure. Extracellular *Glutamine synthetase* is clearly a prime target of the irreversible *Glutamine synthetase* inhibitor l-methionine- *S*-sulfoximine. The inhibitor reduced *Glutamine synthetase* activity in the extracellular milieu of *M. tuberculosis* cultures by 80% but had little effect on cell-associated *Glutamine synthetase*[38].

2.Marcus A. Horwitz *et al.,* assessed the role of *Glutamine synthetase* (GS), in the pathogenicity of *Mycobacterium tuberculosis*; glnA1 was constructed via allelic exchange. The mutant had no detectable *Glutamine synthetase* protein or *Glutamine synthetase* activity and was auxotrophic for L-glutamine. In addition, the mutant was attenuated for intracellular growth in human THP-1 macrophages. Based on growth rates of the mutant in the presence of various concentrations of L-glutamine the importance of the enzyme was known. These studies demonstrate that glnA1 is essential for *M.tuberculosis* virulence[39].

3.Lukasz Berlicki studied about the *Glutamine synthetase* enzyme which catalyses the formation of glutamine from glutamine and ammonium ion. It is one of the most important enzymes in nitrogen metabolism. The first part of the review presents the long-dating research on inhibitors of *Glutamine synthetase*. The second part of the paper is dedicated to potential medical applications of *Glutamine synthetase* inhibitors, which is proved as effective anti-tuberculosis agent with high selectivity towards the pathogen[40].

4.O Lagerlund synthesized some potential anti-tubercular agents which targeted *Glutamine synthetase* (GS), which is one of the latest targets of *M.tuberculosis* which catalyses the formation of glutamine from glutamic acid. In this work, novel *Glutamine synthetase* inhibitors and new Pd (O) - catalyzed methods have been developed[41].

5.Wojciech W. Krajewski *et al*., summarized that *Glutamine synthetase* catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP. The enzyme is a central component of bacterial nitrogen metabolism and is a potential drug target. This study provides the first reported structure for a tauto form of the tuberculosis enzyme.

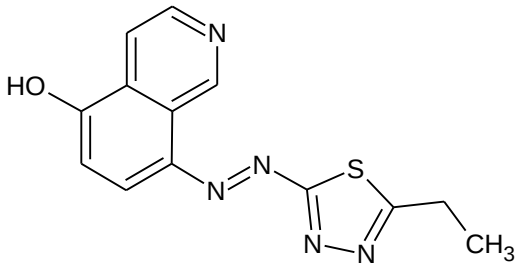
The phospho compound, generated in situ by an active enzyme, mimics the phosphorylated tetrahedral adduct at the transition state. Some differences in ligand interactions of the protein with phosphorylated compound and nucleotide were observed compared with the earlier structures; a third metal ion also was found[42].

The review on following works provided ideas about the Thiadiazole Nucleus and its Biological Activity.

Antimicrobial activity:-

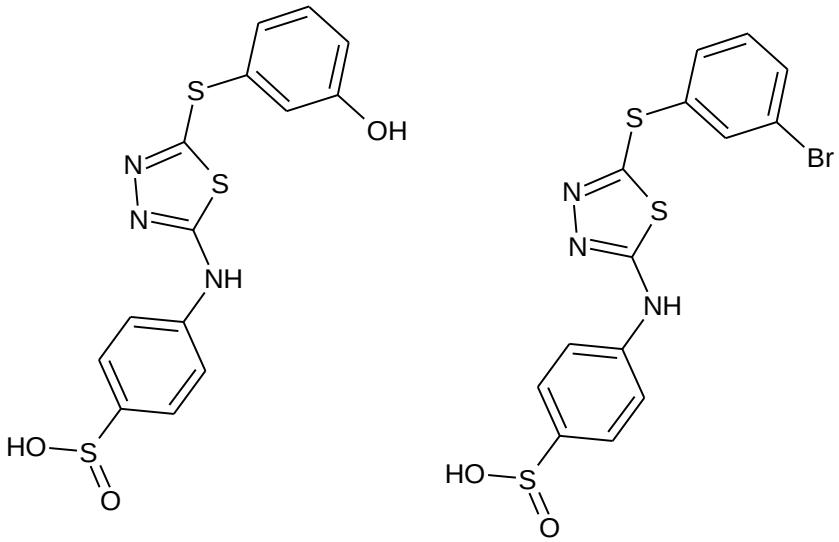
6.Kumar, et al., (2011), synthesized the heterocyclic azodyes derived from thiadiazole and evaluated them for antimicrobial activity. In this study 5-ethyl-1, 3, 4- thiadiazole-2-amine was synthesized by a single step reaction. A series of heterocyclic azodyes were synthesized by coupling 8-hydroxyquinoline, 2, 6-diaminopyridine, *N, N*- dimethyl aniline, 2-napthol and resorcinol with diazotized 5-ethyl-1, 3, 4-thiadiazol-2-amine in

nitrosyl sulphuric acid. The synthesized compounds were also screened for biological activity and showed maximum activity[43].



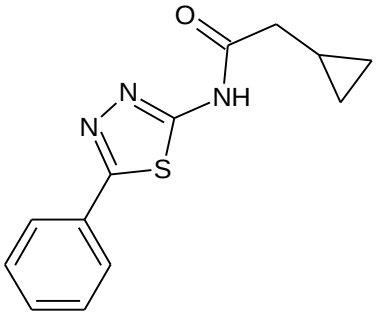
Anti inflammatory and analgesic activity:-

7.Sainy et al., (2009), synthesized a series of 2- amino-5-sulfanyl-1, 3, 4-thiadiazole derivatives and several 2-amino-5-sulfanyl-1, 3, 4- thiadiazoles and concluded that the compounds were associated with lesser degree of anti- inflammatory activity when compared to indomethacin. Only compound 4-[5-(4- Fluorophenylsulfanyl) -[1,3,4] thiadiazol-2-ylamino] benzene sulfonamide showed 65.90% inhibition of paw edema after 3 h at 56 mg/kg (body weight) dose and 66.40% protection in acetic acid induced inflammation in mice[44].



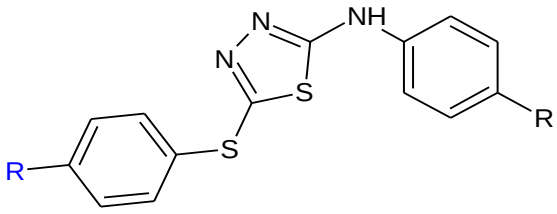
Antifungal Activity:-

8.Liu *et al*., (2011), reported the antibacterial and antifungal activity of 1,3,4-thiadiazoles bearing imidazo [2,1-b] thiazole moiety against *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *T. tonsurans* NCPF245 with MIC of 64, 32, and 8 μg/ml, respectively. Applying QSAR study, it has been observed that positions-2 or position-3 of benzene attached with thiadiazole ring where as electron- donating and bulky group would be favorable for higher antifungal activity[45].



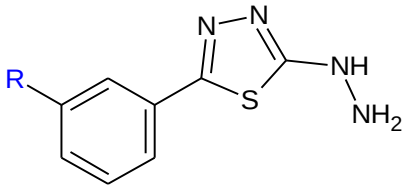
Anticonvulsant activity:-

9.Sharma et al., (2011), synthesized a new series of 2-amino-5- sulfanyl-1, 3, 4-thiadiazole derivatives. All compounds were screened for central nervous system activity. They exhibit significant antidepressant, anxiolytic and anticonvulsant activity when compared with the standard drugs[46].



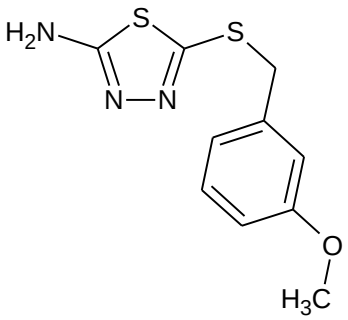
Antihypertensive activity:-

10.Turner et al., (1998), synthesized a series of antihypertensive thiadiazole derivatives and evaluated them for antihypertensive activity and indicated higher activity when compared with the standard compounds[47].



Antidepressant and Anxiolytic Activity:-

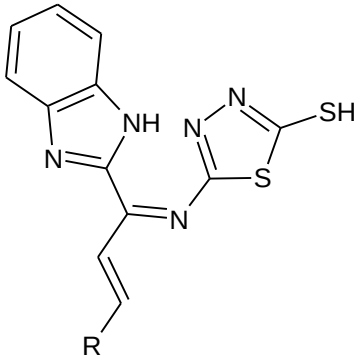
11.Francesca Clerici et al., (2000), A series of 2-amino-5-sulfanyl-1,3,4-thiadiazole derivatives bearing different substituents were synthesized and screened pharmacologically in order to evaluate their central nervous system activity. The purpose of this study was to evaluate the effects of the title compounds on CNS activity by varying the substituents in the thiadiazole moiety. It was found that some of these compounds possess marked antidepressant and anxiolytic properties comparable in efficiency to the reference drugs Imipramine and Diazepam[48].



Anti-Oxidant activity and Mucomembranous protector:-

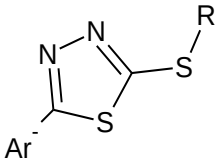
12.Bijo Mathew and Jerad Suresh et al., (2012), A series of some novel imines of 2-amino, 5-thio 1,3,4-thiadiazole connected to benzimidazole chalcones were prepared. Predicted activity spectra of all the final derivatives were determined in the category of

mucomembranous protector nature with a pka value more than 0.7. All the newly synthesized compounds were screened for their antiulcer activity in the pylorus-ligated rats[49].

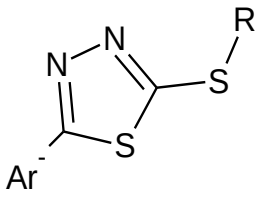


Anti tubercular activity:-

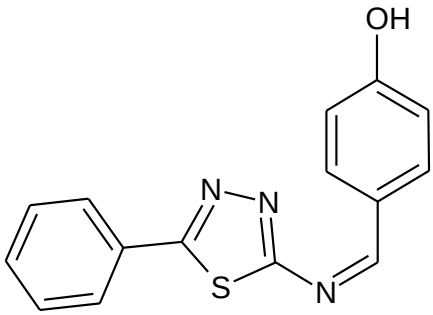
13.Foroumadi et al., (2002), Synthesized 2-(5-nitro-2-furyl) and 2- (1-methyl- 5-nitro-lH- imidazol-2-yl)-1, 3, 4-thiadiazole derivatives and evaluated *in vitro* anti tuberculosis activity. In this study two series of 2-(5-nitro-2-furyl) and 2-(1-methyl-5- nitro-lH-imidazol-2-yl)-5-propyl, ally and propargyl) thio-l,3,4-thiadiazoles derivatives and 2-(5-nitro-2-furyl)- and 2-(1-methyl-5-nitro-lH-imidazol- 2-yl)-5-(nitro benzyl)thio-l,3,4-thiadiazole derivatives were synthesized and evaluated against *Mycobacterium tuberculosis* and found to exhibit good activity[50].



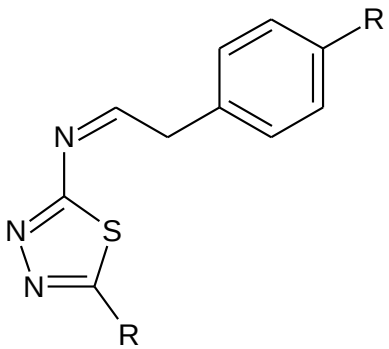
14.Noolvi M.N et al., (2013), synthesized a series of imidazo [2, 1-b] 1, 3, 4- thiadiazole derivatives. The synthesized compounds were evaluated for their invitro anti tubercular activity against *M. tuberculosis* H37Rv strain by using Alamar Blue susceptibility test. Among the tested compounds, 2-(1-methylimidazol-2- yl)-6-(4-nitrophenyl) imidazo [2, 1-b] 1, 3, 4-thiadiazole have shown the highest inhibitory activity with MIC of 3.125µg/ml as compared to other compound[51].



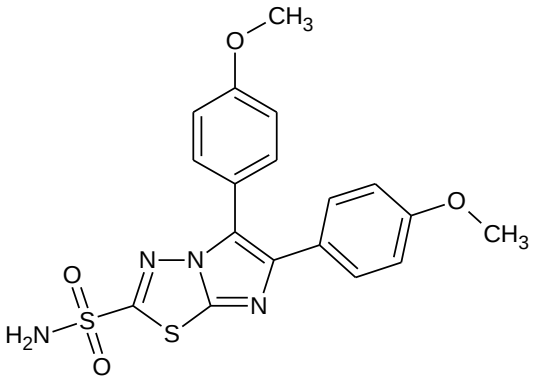
15.Gadad A.K et al., (2004), Evaluated 6-aryl-2-triflouromethylimidazo [2, 1-b] 1, 3, 4-thiadiazole derivatives against *M. tuberculosis* ie. H37Rv strain by radiometric BACTEC and broth dilution method. It was found that 4-flouro phenyl derivative causes maximum inhibition at 6.25µg/ml concentration. All the synthesized compounds were reported to be less active than standard drug Isoniazid[52].



16.Shiradhkar M et al., (2005), synthesized a series of S-triazolo [3, 4-b] 1, 3, 4-thiadiazoles and screened for their antitubercular activity against *M. tuberculosis* H37Rv. The final data of the MIC was compared with the standard drug Rifampicin at 0.03µg/ml concentration which showed more than 95% inhibition. Among the derivatives, nitro phenyl derivatives were shown to possess maximum activity against *M. tuberculosis*[53].

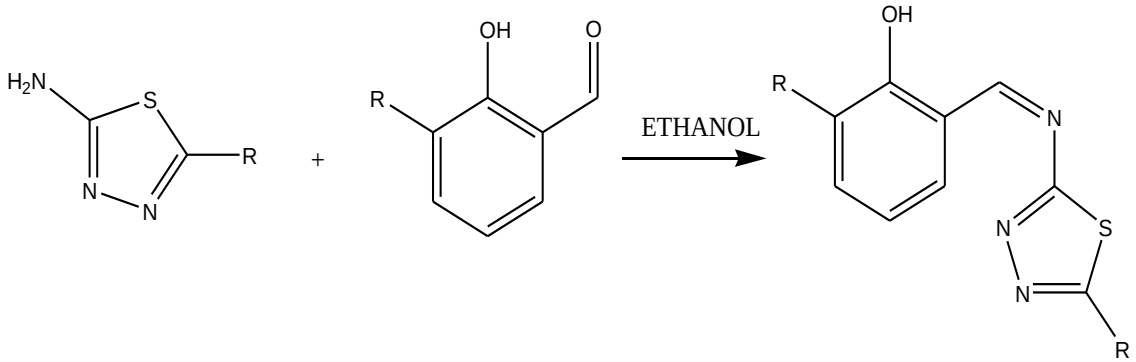


17.Palkar M.B et al., (2012), synthetized a series of anti-tubercular activity of some 2-substituted-5, 6-diaryl substituted imidazo [2, 1-b] 1, 3, 4-thiadiazoles against *M. tuberculosis* H37Rv strain by Micro plate Alamar Blue Assay (MABA) method. Among synthesized compounds, the below represented compound (MIC =1.25 lg/ml) exhibited excellent anti-tubercular activity with respect to other synthesized compounds and reference drugs[54].

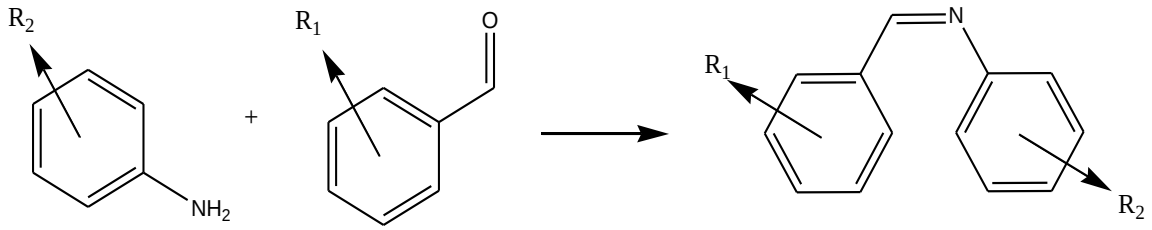


The review on following works provided ideas for synthesis of the desired chemical entities:-

18.Tang, Zilong et al., (2015), synthesized 2-[1, 3, 4-thiadiazolyl amino methyl] phenols by one pot reaction of 2-amino -5-Alkyl [aryl] - 1, 3, 4-thiadiazole and salicylaldehyde. The title compounds had moderate fungicidal activity[55].



19.Sandeep Miglani, et al., (2012), reported the rapid synthesis of Schiff-bases without solvent under microwave irradiation and their antimicrobial activity It was observed that meta-substituted compounds exhibited good activity against almost all the organisms used in the study[56].



The following literature provide supporting data for the drug design study:-

20.Wermuth C G., (2006) reviewed the similarity in drugs with the importance and reflections on analogue design. He also clarified the terminology of analogue design by establishing a clear distinction among three kinds of analogues[57].

21.Kore P P *et al*., (2012) reviewed a brief history of CADD, DNA as target, receptor theory, structure optimization, structure-based drug design, virtual high-throughput screening (vHTS) and graph machines[58].

22.Frederick W G *et al*., (2015) outlined the general principles that should be applied to ensure the building block collection's impact on drug discovery projects[59].

The review on following works revealed the basis of Microplate Alamar Blue Assay for evaluating the anti-mycobacterium action:-

23.David A. J. Moore., et al., (2008), reported inter and intra assay reproducibility of Microplate Alamar Blue Assay results for Isoniazid, Rifampicin, Ethambutol, Streptomycin, Ciprofloxin, Capreomycin, drug susceptibility testing Mycobacterium tuberculosis[60]**.**

24.Vanitha J D., et al., (2007), reported the evaluation of Microplate Alamar Blue Assay for drug susceptibility testing of Mycobacterium avium complex isolates[61].

25.Franzblau S G., et al., (1998), reported the, Rapid lower technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the Micro plate Alamar blue assay. Journal of Clinical Microbiology[62].

26.Jose d Jesus Alba-Romero.,et al., (2014) applied the Microplate Alamar Blue Assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The results showed that the MABA test is fast and easy to apply. It is very reliable method to determining the drug susceptibility to pharmaceuticals[63].

The review on following works revealed the basis of Acute Oral Toxicity on Mice:-

27.Erik Walum.,(1998) gives a short review of methods for acute toxicity testing with the emphasis on the median lethal dose (LD50) test and alternative procedures that fulfill the requirement of reducing, refining, or replacing the use of animals in toxicity testing (the 3R principle). Furthermore, this review mirrors the current discussion on the usefulness of different procedures for acute toxicity testing and surveys the conclusions of various panels, commissions, and groups[64].

28. **Gerald L. Kennedy, (**1986**)** studied the reduction of the numbers of experimental animals used in test programs while minimizing the extent of information. In working with newly synthesized chemicals or adapting chemicals for new end-uses, the definition of the biologic spectra of effects produced includes estimation of the amount necessary to produce biologic signs of response up to and including death. Traditionally, the LD,, was used to obtain this information using laboratory species such as the rat, mouse, guinea pig and hamster[65].

MATERIALS AND METHODS

4. MATERIALS AND METHODS

DRUG DESIGN[66]:-

The molecular designing of drugs for specific purposes (such as DNA-binding, enzyme inhibition, anti-cancer efficacy, etc.) based on knowledge of molecular properties such as activity of functional groups, molecular geometry, and electronic structure, and also on information cataloged on analogous molecules.

Drug design is generally computer-assisted molecular modeling and does not include pharmacokinetics, dosage analysis, or drug administration analysis.

DOCKING STUDY:-

Molecular docking is generally used to detect the protein-ligand orientation and interaction. AutoDock Tools package was utilized to create the docking input files. The grid region was surrounded by the active site for binding. Docking of ligands is carried out by Autodock 4® software.

Docking allows the medicinal chemist to virtually screen a set of compounds and predict the strongest binding capacity based on various scoring function. It explores ways in which two molecules such as ligand and receptor (protein) fit together and docks to each other well. The molecule binding to a receptor inhibits its function and thus acts as drug.

Working with AutoDock 4® includes 3 steps:

1. Preparation of receptor & ligand files.
2. Calculation of affinity maps by using a 3D grid around the receptor & ligand.
3. Defining the docking parameters and running the docking simulation.

The preparation step starts with pdb files of receptor (protein.pdb) and ligand (ligand.pdb), to which are added hydrogens and then saved as protein.pdb & ligand.pdb. The calculation of affinity maps in the "Grid" section requires the above pdb files to be assigned charges & atom types, and also that the nonpolar hydrogens are merged. This is done automatically by AutoDock tools, and the resulting files need to be saved as

protein.pdbqt & ligand.pdbqt, which is the only format AutoGrid & AutoDock can work with. Calculation of affinity maps is done by AutoGrid, and then docking can be done by AutoDock. The newest docking algorithm is LGA (Lamarckian Genetic Algorithm).

For running AutoDock tools, the following steps are required.

1. Install MGL (**Molecular Graphics Laboratory**) tools.
2. Download **autogrid4.exe**, **autodock4.exe** and **AD4.1_bound.dat** files.
3. Install **Molegro Molecular Viewer**®.
4. Install **ChemBioDraw**® including **3D Pro**.

For docking the Enzyme and Ligand is needed. The enzyme which is downloaded from (**Research Collaboratory for Structural Bioinformatics**) RCSB Protein Data Bank as PDB format. The **Protein Data Bank** (PDB) is a crystallographic database for the three-dimensional structural data of large biological molecules, such as proteins and nucleic acids. The data, typically obtained by X-ray crystallography, NMR spectroscopy, or, increasingly, Cryo-electron microscopy, and submitted by biologists and biochemists from around the world, are freely accessible on the Internet via the websites of its member organisations (PDBe, PDBj, and RCSB).

Criteria to select the best protein structure from PDB[67]:-

1. Resolution must be minimum possible (This will ensure the better quality of protein structure).

2. Domain completeness. The PDB structure is examined and confirmed that the under study domain's full structure available. Partial domain will lead to false interpretations.

3. Variant /Mutations. We need to look for whether the structure is a wild type or a mutant/variant. In case of a mutant structure requirement we may have to introduce required mutations manually and model them.

4. Side Chain Completeness (is of secondary importance). Structures determined through old techniques might have (Not always) missing side chains due to flaw in tech or manual error. Right 3D confirmation of side chains is critical in small

ligand binding thus ensuring their completeness is important. As a possible solution we may look for latest structure availability of the same.

5. Ligand /Crystalline Water / Co factor presence. To get out the right docking result removal (As per case study) of these elements form PDB file is important.

According to the above criteria the *Glutamine synthetase I* enzyme **3zxr** as pdb format was downloaded which has a X-ray resolution of 2.15 A^o and the structure was detected by X-ray Diffraction method.

After having finished installation of above tools, we can run AutoDock Tool.

1. Create a work folder for the job and tag it based any name.

2. Running ADT in start menu in windows: > after running we should set its directory by the following steps:

Open ADT> File> Preferences>set> Startup Directory (in this section you should put on the workfolder path and set it).

3. Prepare ligand and protein in ".pdb" format. Notice, we must have labeled them by "Ligand" and "Protein" keywords. Protein can be preprocessed by Molegro Molecular Viewer® & Ligand can be initially prepared using in ChemBioDraw® and can be saved as pdb.

Preparation of Protein:

1. File > Read Molecule> Select Protein File (“.pdb” file).

2. Edit > Hydrogens >Add>Polar Only >OK.

3. Edit > Hydrogens > Merge Non-Polar > Continue (if warning appears in this step please click on "continue").

4. Edit > Charges > Compute Gasteiger > OK.

5. Edit > Misc > Repair Missing Atoms > Ok/Select all > Dismiss.

6. File > Save > Write PDB> >Sort Nodes (Check) > OK > (Overwrite) YES.

Preparation of Ligand:

1. Ligand > Input > Open> Select Ligand File (".pdb" file) > OK.
2. Edit> Charges> Add Kollman charges.
3. Ligand > Output > Save as PDBQT.

Executing AutoGrid4: (Preparing gpf file)

1. Grid > Macromolecule > Choose > Click (Protein) > Select Molecule > OK > Save as PDBQT.
2. Grid> Set Map types > Choose Ligand > Click 'Ligand' > Select 'Ligand'.
3. Grid > GridBox> Set the BOX to comprise the active aminoacid of the macromolecule > Center> Pick an atom> Center> Center on Macromolecule > File > Close Saving Current.
4. Grid > Output > Save GPF> grid.gpf> save.

Executing AutoDock4: (Preparing dpf file)

1. Docking > Macromolecule > Set Rigid filename> Select 'Protein.pdbqt' > Open.
2. Docking > Ligand > Choose> Click 'Ligand' > Select 'Ligand' > Accept.
3. Docking > Search Parameters > Genetic Algorithm> Accept.
4. Docking > Output > Lamarckian GA> Save file as 'dock.dpf'.

The autogrid4.exe, autodock4.exe and AD4.1_bound.dat files are copied into the destination folder (working folder) and checked whether pdbqt file, grid.gpf and dock.dpf files are there.

1. Open cmd (command prompt). Windows +R and type cmd enter.
2. In command prompt, the destination folder is directed by cd commands.
3. Run the following commands.
4. Autogrid4.exe -p grid.gpf -l grid.glg (wait for the response).
5. Autodock4.exe -p dock.dpf -l dock.dlg wait for the response).
6. The dock.dlg file is the docking result.

Analysing the Results: (Screening)

1. Analyze > Docking > Open> Select 'dock.dlg' > Open >Assign Ligand New Name > OK.

2. Analyze> Macromolecule > Choose > Click'Protein'>Select Macromolecule.

3. Analyze > Conformations > Play, Ranked By Energy or Play > Click on the '&' Button.

4. Set Play Options > Check 'Build H-Bonds' > View the hydrogen bonds formed > Check 'Show Info'> View the Interaction Energy > Build Current Write Complex > Save as 'Result.pdb'.

5. For better ligand interactions open the result.pdb in Molegro Molecular Viewer® and view it and save the ligand interaction as image.

During the docking process, a maximum of 10 conformers are considered for each compound. This method was applied for each designed compound and after completion the conformer with lowest binding energy was chosen.

The conformational similarity by visualizing the binding site and its energy (Kcal/mol), and the docked amino acid residues forming hydrogen bonds and other parameters like intermolecular energy (Kcal/mol) and inhibition constant (μM) were analyzed by AutoDock tool. Ten best poses were generated for each ligand and scored using AutoDock 4® scoring functions. Based on the docked energy all the ligands were ranked. The ligand interacting residues with the target protein were analyzed using Molegro molecular Viewer®.

LIPINSKIS RULE[68,69]:-

Lipinski's rule of five is a rule of thumb to evaluate drug likeness, or to determine if a chemical compound with a certain pharmacological or biological activity has the properties that would make it a likely orally active drug in humans. The rule was formulated by Christopher A. Lipinski in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion

(ADME). However, the rule does not predict if a compound is pharmacologically active.

The rule is important for the drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds, and a higher lipophilicity.

Lipinski's rule says that, an orally active drug has no more than one violation of the following criteria:

1. Not more than 5 Hydrogen bond donors (Nitrogen or Oxygen atoms with one or more Hydrogen atoms)
2. Not more than 15 rotatable bonds.
3. Not more than 10 Hydrogen bond acceptors (Nitrogen or Oxygen atoms)
4. Molecular weight under 500 Daltons
5. Partition coefficient of log P less than 5.

Variants

In an attempt to improve the predictions of druglikeness, the rules have spawned many extensions,

1. Partition coefficient log P in −0.4 to +5.6 range
2. Molar refractivity from 40 to 130
3. Molecular weight from 180 to 500
4. Number of atoms from 20 to 70 (includes H-bond donors [e.g. OHs and NHs] and H-bond acceptors [e.g. Ns and Os])

Also the 500 molecular weight cutoff has been questioned. Polar surface area and the number of rotatable bonds has been found to better discriminate between compounds that are orally active and those that are not for a large data set of compounds in the rat.

In particular, compounds which meet only the two criteria of:

1. 10 or fewer rotatable bonds and
2. Polar surface area no greater than 140 A^2

are predicted to have good oral bioavailability.

INSILICO PREDICTION[37]:

The synthesized molecules are predicted of their toxicity risk and some significant physicochemical properties were performed using OSIRIS-Property-Explorer. It is a free tool to predict physico-chemical and toxicological molecular properties, which need to be optimized when designing pharmaceutically active compounds. For predicting properties of a chemical compound just draw its structure and Property Explorer will start calculating properties as soon as a chemical structure is valid. Charges should be balanced and atom valances not exceeded. Nitro-groups, for instance, should be drawn with a positive charge on nitrogen and a negative on one of the oxygens with a single bond connecting these two atoms. For an explanation of the significance of a specific property and the calculation algorithm applied see the corresponding page below:

1. Molecular Weight
2. Calculated LogP
3. Aqueous Solubility
4. TPSA
5. Drug-Likeness
6. Toxicity Assessment
7. Overall Drug-Score

It also shows the toxicological predictions such as Mutagenic, Tumorigenic, Irritant and Reproductive effect.

Property Explorer Applet predicts physico-chemical properties and detects potential toxicity risks for any drawn chemical structure in real time. Prediction results are valuated and color coded. Unfavorable properties or those with a high risk of side effects like mutagenicity or poor intestinal absorption are shown in red. Green color instead indicates drug-conform behaviour.

MOLINSPIRATION®:

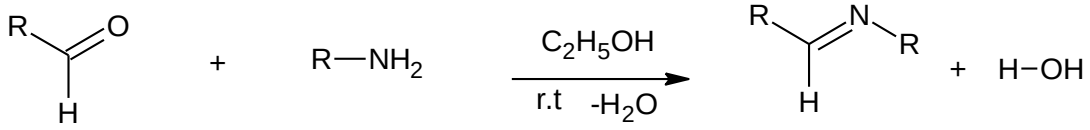
Molinspiration® is an online tools to calculate the molecular properties including PSA, Rule of 5 parameters and molecular drug-likeness by drawing the structure of the docked molecule by the use of online provided tools.

SYNTHETIC METHODOLOGY[70,71]:

The designed and docked compounds can be synthesized by the following scheme (Schiff base synthesis) which is presented below.

In a round bottom flask, equimolar mixture of an aldehyde or ketone and amine in ethanol is taken. The resulting mixture is refluxed for about 4-5 hours at 60 to 80°C temperature. The progress of the reaction is monitored by TLC. The mixture is poured on to crushed ice. The precipitate obtained is filtered by whatmann filterpaper. The pure product is obtained upon recrystallization of the residue from ethanol, when necessary. All the products are known imines and their structures were secured on the basis of their analytical and/or spectral data, compared with literature data.

SYNTHETIC SCHEME[72]:

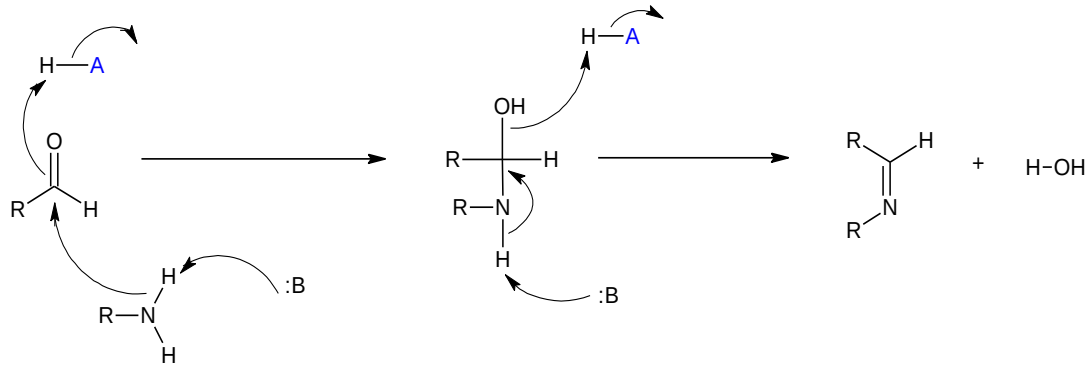


Substituted Aromatic Aldehyde

Substituted Aromatic Primary Amine

Schiff Base

REACTION MECHANISM[73]:



MATERIALS

Substituted Aromatic Primary Amine :

5-AMINO-1,3,4-THIADIAZOLE-2-THIOL

Substituted Aromatic Aldehydes :

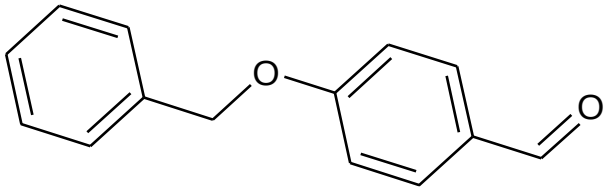
1. 4-(BENZYLOXY) BENZALDEHYDE
2. CINNAMALDEHYDE
3. 3-NITRO BENZALDEHYDE
4. 2-HYDROXY BENZALDEHYDE
5. 4-HYDROXY BENZALDEHYDE

REACTANT PROFILE:

5-AMINO-1,3,4-THIADIAZOLE-2-THIOL:

Synonym	: 2-Amino-1,3,4-Thiadiazole-5-Thiol
Molecular Formula	: $C_2H_3N_3S_2$
Molecular weight	: 133.195
Description	: White or Pale yellow to cream powder
Melting point	: 235^oC
Solubility	: Slightly in Methanol, Sparingly in Chloroform

4-(BENZYLOXY) BENZALDEHYDE:



Synonym : 4-(PhenylMethoxxy) Benzaldehyde

Molecular Formula : $C_{14}H_{12}O_2$

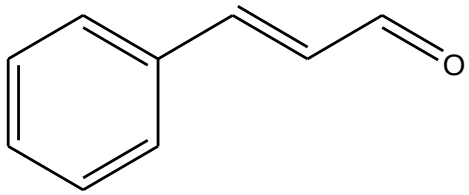
Molecular weight : 212.244

Description : Creamish to Yellow crystalline powder

Melting point : 71-74^oC

Solubility : Slightly in Chloroform, Sparingly in Ethyl acetate

CINNAMALDEHYDE:



Synonym : 3-Phenylpropenal

Molecular Formula : C_9H_8O

Molecular weight : 132.159

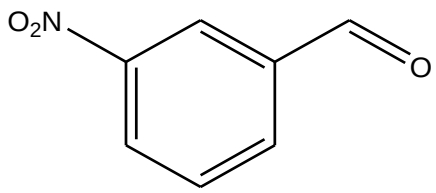
Description : Pale Yellow viscous liquid

Boiling point : 250-252^oC

Solubility : Soluble in Ether and Chloroform

Density : 1.05 g/ml at 25^oC

3-NITRO BENZALDEHYDE:



Synonym : m-Nitro Benzaldehyde

Molecular Formula : $C_7H_5NO_3$

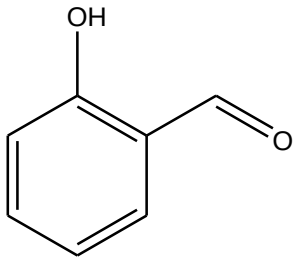
Molecular weight : 151.119

Description : Yellowish to Brownish crystalline powder to granulate

Melting point : 58.5^oC

Solubility : Slightly in water

2-HYDROXY BENZALDEHYDE:



Synonym : Salicylaldehyde, o-Hydroxy Benzaldehyde

Molecular Formula : $C_7H_6O_2$

Molecular weight : 122.121

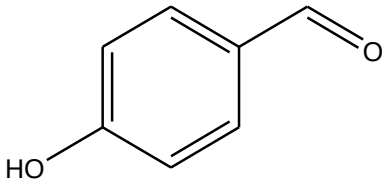
Description : Clear Yellow liquid

Boiling point : 197^oC

Solubility : Slightly in water

Density : 1.146 g/ml at 25^oC

4-HYDROXY BENZALDEHYDE:

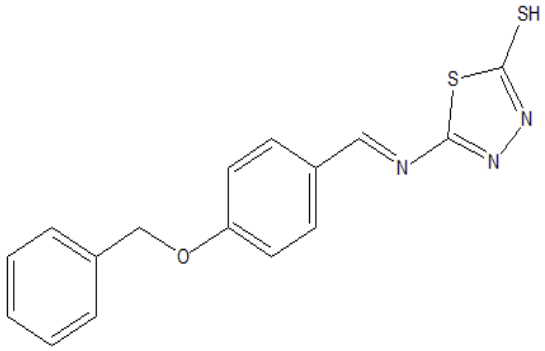
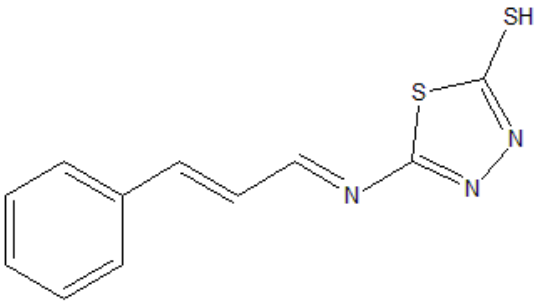


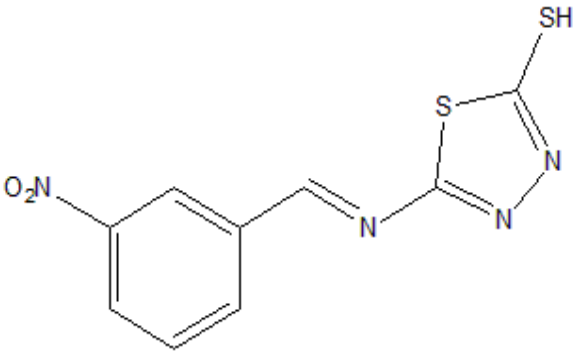
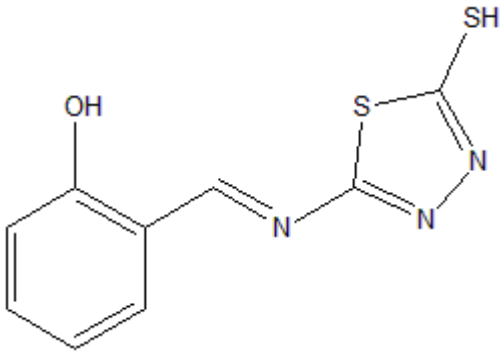
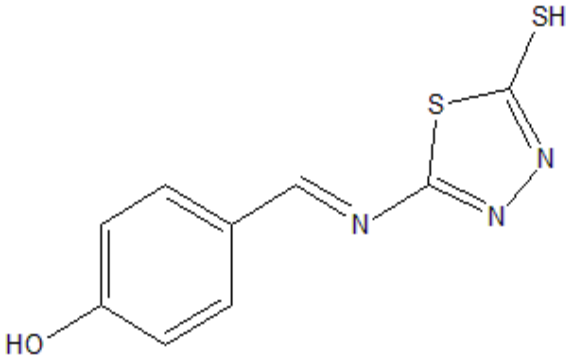
Synonym	: p-Hydroxy Benzaldehyde
Molecular Formula	: $C_7H_6O_2$
Molecular weight	: 122.121
Description	: Light Yellow to Light Brown crystalline powder
Melting point	: 112-116^oC
Solubility	: soluble in water and Ethanol

List of Compounds to be Synthesized:

The **5-Amino-1,3,4-Thiadiazole-2-Thiol** an amine can be reacted with following aldehydes such as 4-(Benzyloxy) Benzaldehyde, Cinnamaldehyde, 3-Nitro Benzaldehyde, 2-Hydroxy Benzaldehyde, 4-Hydroxy Benzaldehyde separately and to form corresponding Schiff bases.

Table 2. List of compounds to be synthesized

Sample code	Structure of the Molecule	IUPAC Name
BOB		5 - ({(*E*) - [4-(benzyloxy)phenyl] methylidene} amino)-1,3,4-thiadiazole-2-thiol
CIN		5 - {[(1*E*, 2*E*) -3-phenylprop-2- en-1-ylidene] amino}-1,3,4-thiadiazole-2-thiol

Sample code	Structure of the Molecule	IUPAC Name
NIB		5 - {[(*E*) - (3-nitrophenyl) methylidene] amino}-1,3,4-thiadiazole-2-thiol
OHB		2 - {(*E*) - [(5-sulfanyl-1,3,4-thiadiazol-2-yl) imino] methyl}phenol
PHB		4 - {(*E*) - [(5-sulfanyl-1,3,4- thiadiazol-2-yl) imino]methyl}phenol

CHARACTERIZATION METHODS:

- TLC method
- Melting point.
- Infrared Spectroscopy.
- LC-Mass Spectroscopy
- H^1 Nuclear Magnetic Resonance Spectroscopy.

Thin Layer Chromatography:(Ascending Technique)

Precoated TLC plates with Silica Gel GF 250 are used. Samples of Reactants and Products are prepared with suitable solvents. The plates are cutted to optimum size for spotting the samples. Then the TLC development chamber was saturated with suitable mobile phase (n-Hexane: Methanol: Ethyl acetate) of different ratios for 15 to 20 min. After saturation the samples are spotted carefully on the TLC plates. Then the plates are kept inside the chamber for elution of the sample. After elution the plates are removed from the chamber and dried. Then the plates are viewed under UV chamber by Physical method or Chemical method by Iodine chamber. If the single spot was observed indicating the completion of the reaction. The R_f was calculated by the following formula,

R_f = Distance travelled by the solute / Distance travelled by the solvent

The different mobile phase was used to confirm the completion of the reaction.

Melting Point:

The physical properties of a compound, such as melting point and boiling point can provide useful information which can help in the identification of a sample or to establish its purity. The temperature at which a solid melts and becomes a liquid is the melting point. Since this requires that the intermolecular forces that hold the solid together have to be overcome, the temperature at which melting occurs will depend on the structure of the molecule involved - an example of the relationship between structure and properties. Hence, different compounds tend to have different melting points.

A pure, nonionic, crystalline organic compound usually has a sharp and characteristic melting point (usually 0.5-1.0^{o}C range). A mixture of very small amounts of miscible impurities will produce a depression of the melting point and an increase in the melting point range. Consequently, the melting point of a compound is a criterion for purity as well as for identification.

The melting point of an organic solid can be determined by introducing a tiny amount into a small capillary tube, attaching this to the stem of a thermometer centred in a heating bath, heating the bath slowly, and observing the temperatures at which melting begins and is complete.

Infra-Red Spectroscopy:

IR (region 2.5-15 μ) is a powerful tool for identifying pure organic and inorganic compounds because. With the exception of a few homo nuclear molecules such as O_2, N_2, C_{14} all the molecular species absorb infrared radiation. With the exception of chiral molecules in the crystalline state, each molecular species has a unique infrared absorption spectrum.

Mass Spectroscopy:

Mass spectroscopy is an analytical techniques used to establish the molecular structure and the molecular weight of the analyte under investigation. In this technique, the compound under investigation is bombarded with a beam of electrons producing ionic fragments of the original species. The relative abundance of the fragment ion formed depends on the stability of the ion and of the lost radical. The resulting charged particles are then separated according to their masses. Mass spectrum is a record of information regarding various masses produced and their relative abundances.

Liquid Chromatography–Mass Spectrometry (**LC-MS**) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography (or HPLC) with the mass analysis capabilities of mass spectrometry (MS). Coupled chromatography - MS systems are popular in chemical analysis because the individual capabilities of each technique are enhanced synergistically. While liquid

chromatography separates mixtures with multiple components, mass spectrometry provides structural identity of the individual components with high molecular specificity and detection sensitivity. This tandem technique can be used to analyze biochemical, organic, and inorganic compounds commonly found in complex samples of environmental and biological origin. Therefore, LC-MS may be applied in a wide range of sectors including biotechnology, environment monitoring, food processing, and pharmaceutical, agrochemical, and cosmetic industries.

H^1 Nuclear Magnetic Resonance Spectroscopy:

Nuclear magnetic resonance involves the interaction between oscillating magnetic field of electromagnetic radiation and the magnetic energy of the hydrogen nucleus or some other type of nuclei when these are placed in an external static magnetic field.

NMR enables us to study the number of equivalent protons and their electronic environment. It reveals the different chemical environment in which the proton is present and helps us to ascertain the structure of molecule.

The number of signals in an NMR spectrum denotes the number of the set of equivalent protons in a molecule. The position of the signals in the spectrum helps us to know the nature of protons such as aromatic, aliphatic, acetylenic, vinyl, adjacent to some electron attracting or electron releasing group etc. the splitting of the signal is due to the different environment of the absorbing proton with respect to the adjacent protons and not with respect to electrons.

BIOLOGICAL EVALUATION:

IN-VITRO ANTI – TUBERCULAR ACTIVITY BY MICROPLATE ALAMAR BLUE ASSAY.

1) The anti mycobacterial activity of compounds were assessed against M. tuberculosis using microplate Alamar Blue assay (MABA).

2) This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with propotional and BACTEC radiometric method.

3) Briefly, 200µl of sterile deionzed water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

4) The 96 wells plate received 100 µl of the Middlebrook 7H9 broth and serial dilution of compounds were made directly on plate.

5) The final drug concentrations tested were 100 to 0.2 µg/ml.

6) Plates were covered and sealed with parafilm and incubated at 37ºC for five days.

7) After this time, 25µl of freshly prepared 1:1 mixture of Almar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 hrs.

8) A blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth.

9) The MIC was defined as lowest drug concentration which prevented the color change from blue to pink.

Standard Strain used: ***Mycobacteria tuberculosis*** (Vaccine strain, H37 RV strain): ATCC No- 27294.

Standard values for the Anti-Tb test which was performed.

Pyrazinamide- 3.125µg/ml

Ciprofloxacin-3.125µg/ml

Streptomycin- 6.25µg/ml

ACUTE ORAL TOXICITY STUDY:

Acute oral toxicity study (Limit Test) was designed as per the OECD guidelines (423).

Principles and purpose

The main purpose of acute toxicity is to evaluate the degree of toxicity in a quantitative and qualitative manner.

Experimental Animals

Six healthy adult Albino mice were weighing between 20-25g were selected for the study. For all the six animals food, but not water was withheld overnight prior to dosing.

Selection of dose levels and administration of dose:

Being synthetic molecules, the mortality was unlikely at the highest starting dose level (2000mg/kg/b.w). Hence a limit test one dose levels of 2000mg/kg/b.w was conducted in all animals as per the OECD guidelines (423).

Procedure:

Animals are observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. However, the duration of observation should not be fixed rigidly. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

CYTOTOXICITY EVALUATION[77]:

Material:

Cell line: Vero (African green monkey kidney cells), DMEM, Fetal Bovine Serum (FBS), antibiotic–antimicotic solution and SRB reagent Tissue culture flasks, 96 well micro culture plates.

METHODS:

Maintenance of cell lines:

Vero (African green monkey kidney cells), cell line were grown in 25 cm^2 tissue culture flasks containing DMEM medium supplemented with 10% FBS, 1% L-glutamine and 1% antibiotic-antimicotic solutions at 37ºC in CO_2 incubator in an atmosphere of humidified 5% CO_2 and 95% air. The cells were maintained by routine sub culturing in 25 cm^2 tissue culture flasks.

Sub culturing process of cell lines:

- The culture media from the flasks containing monolayer culture was aspirated and washed with sterile phosphate buffered saline (PBS).
- To the flasks, 1 ml of 0.2% trypsin-EDTA solution was added and after few seconds it was aspirated and flask was kept in incubator 2-3 min. for detachment.
- The flasks were removed from the incubator and gently tapped to detach all the adhering cells. The cell detachment was confirmed by observing under an inverted microscope (Nikon Eclipse TE 2000-5, Japan).
- Once the cells were completely detached from the flasks, 2-3 ml of DMEM media containing 10% FBS was added and mixed well.
- From the stock cell suspension, 1 x 10^5 viable cells/ml suspended in media were seeded in 25cm^2 tissue culture flask containing about 4ml of fresh media and incubated until the flasks attained 60-70% confluence.

Trypsinization:

To obtain a single cell suspension from a monolayer culture, cells were dislodged from the culture flasks by trypsinization.

- From a 60-70% confluent flask, the culture media was aspirated out using a micropipette.
- Cells were washed with 3 ml of PBS to remove trace amount of media.
- To each culture flask 1 ml of trypsin-EDTA was added and after few seconds it was aspirated and the flask was kept in the incubator for 3-4 min for cell detachment.
- Culture flasks were observed under an inverted microscope (Nikon Eclipse, Japan) to ensure that cells were completely dislodged.
- Trypsin activity was stopped by adding 2-3ml media containing 10% FBS.

Seeding:

Exponentially growing cell lines were harvested from 25cm^2 Tissue culture flask and a stock cell suspension (5X10^6 cell/ml) was prepared.

- A 96-well flat bottom tissue culture plate was seeded with 5 x10^3 cells in 0.1 ml of DMEM medium supplemented with 10% FBS and allowed to attach for 24hrs.

Preparation of drug dilutions: (serial dilution)

- 50mg/ml stock solution is prepared using 100% DMSO solution. From this stock solution various final concentrations (viz. 62.5, 125, 250 and 500 µg/ml) of test compound solution was prepared as follows:
- 500 µg/ml: 10 µL sol. was taken from stock and to this 990 µl media was added.
- 250 µg/ml: From 500 µg/ml 500 µl was taken and diluted with 500 µl with media.
- 125 µg/ml: From 250 µg/ml 500 µl was taken and diluted with 500 µl with media.
- 62.5 µg/ml: From 125 µg/ml 500 µl was taken and diluted with 500 µl with media.
- After 24 hrs of incubation, cells were treated with 100 µl of test solutions from respective above stocks and the cells were incubated for 48 hrs.
- The cells in the control group received only the medium containing the 0.5, 0.1 % DMSO.
- Each treatment was performed in triplicates.

SRB Assay:

Principle

Sulforhodamine B (SRB) assay was developed in 1990 is one of the most widely used methods for *in vitro* cytotoxicity screening. The assay relies on the ability of SRB to bind to protein components of cells that have been fixed to tissue-culture plates by trichloroacetic acid (TCA). SRB is a bright-pink amino xanthene dye with two sulfonic groups that binds to basic amino-acid residues under mild acidic conditions and dissociate under basic conditions. As the binding of SRB is stoichiometric, the amount of dye extracted from stained cells is directly proportional to the cell mass.

Reagents:

- 10% (wt/vol) TCA
- 1% (vol/vol) acetic acid
- 0.057% (wt/vol) SRB (Sigma, cat. no. 86183) in 1% (vol/vol) acetic acid
- 10 mM unbuffered Tris base solution

Cell fixation and staining:

- 100 µl ice cold 10% (wt/vol) TCA was gently added to each well and the plates at 4 °C for 1 h.
- The plates were washed four times under slow-running tap water and excess water was removed by air drying the plates at room temperature.
- 100 µl 0.057% (W/V) SRB was added to the wells and incubated for 30 minutes at room temperature in dark.
- The plates were then washed with 1% acetic acid to remove the unbound dye. The plates were air dried at room temperature.
- 200 µl of Tris base (10mM) was added to the wells and plates were places on shaker for 10 min.
- Optical density was read at 540 nm in a ELISA plate reader. Percentage cell death was calculated (absorbance of control wells- absorbance of test wells / absorbance of control wells) x 100.
- IC50 was calculated from the % cell death using GraphPad Prism 7® software.

RESULTS AND DISCUSSION

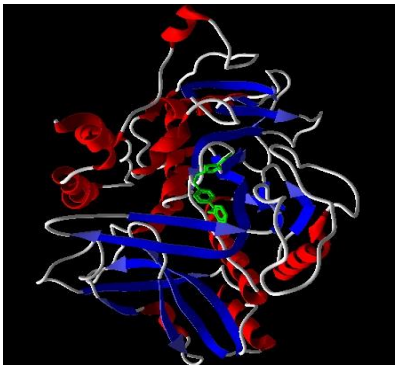
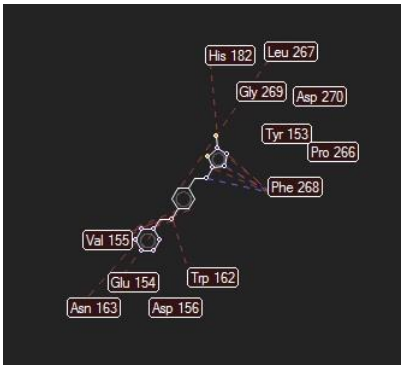
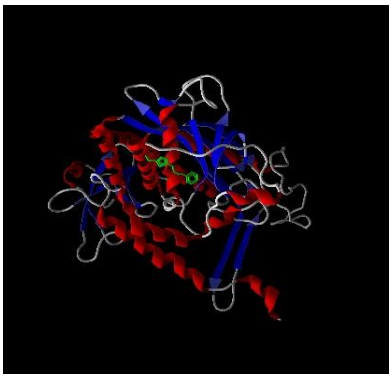
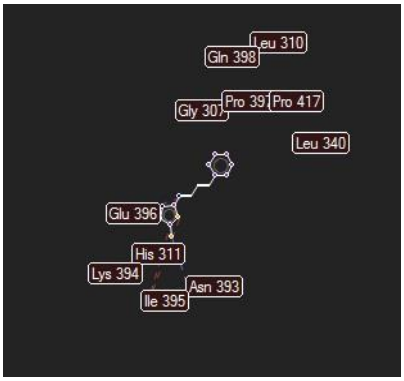
RESULT AND DISCUSSION

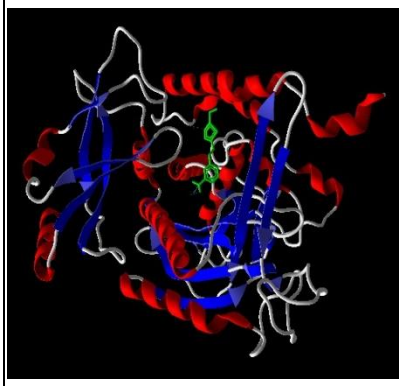
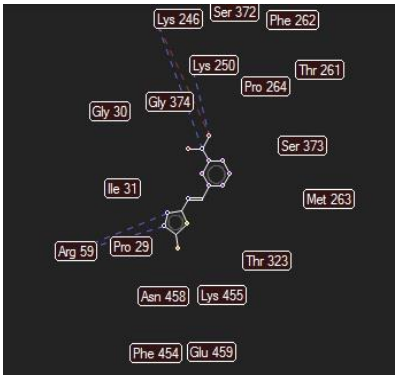
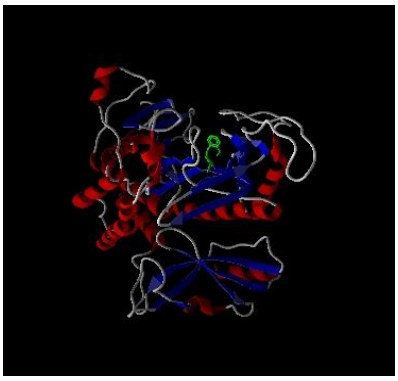
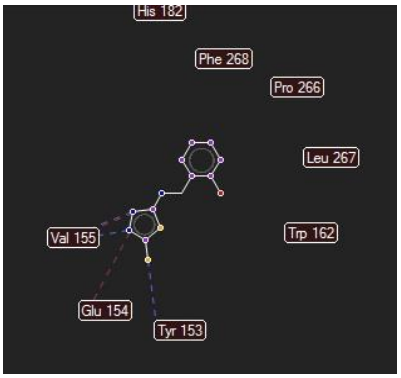
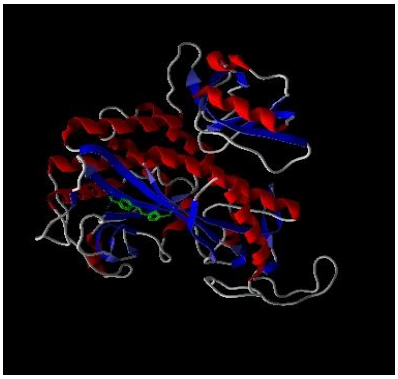
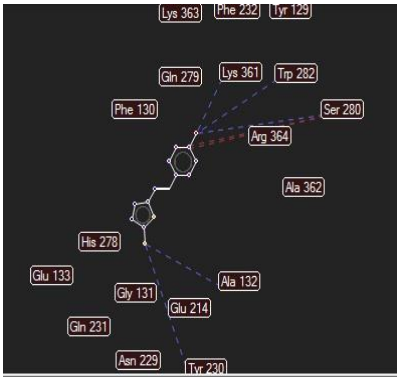
Drug Design:

Docking and Interaction with Target Enzyme:

Around hundred molecules, which were sketched using ChemSketch®, were docked against the *M. tuberculosis*, *Glutamine synthetase I* enzyme using Autodock 4® software. The molecules with best docking score and good interactions were selected and synthesized.

Table 3. Docking and Interaction View

SAMPLE CODE	DOCKING SCORE (K.Cal/mol)	DOCKING VIEW WITH *Glutamine synthetase I* (3zxr)	INTERACTION VIEW *Glutamine synthetase I* (3zxr)
BOB	-6.26		
CIN	-5.32		

SAMPLE CODE	DOCKING SCORE (K.Cal/mol)	DOCKING VIEW WITH *Glutamine synthetase I* (3zxr)	INTERACTION VIEW *Glutamine synthetase I* (3zxr)
NIB	-5.73		
OHB	-4.18		
PHB	-6.73		

By docking with *Glutamine synthetase* enzyme the designed molecules showed better docking score. Among them BOB and PHB molecules have the lowest binding score of -6.26, and -6.73 Kcal/mol which is comparatively better than CIN, NIB, and OHB.

PREDICTION OF DRUG LIKENESS:

Cheminformatics software **Molinspiration**® was employed to evaluate drug likeness. This tool is quick and easy to use. It is a software available online for calculation of important molecular properties like logP, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioavailability score for the compounds with best scoring.

Table 4. Insilico prediction of Drug likeness results

DRUG LIKENESS PARAMETERS	SAMPLE CODE				
	BOB	**CIN**	**NIB**	**OHB**	**PHB**
Log P	4.36	3.46	2.64	2.64	2.23
TPSA	47.38	38.15	83.97	58.38	58.38
No. of non-Hydrogen atoms	22	16	17	15	15
Molecular Weight	327.43	247.35	266.31	237.31	237.31
No.of Hydrogen bond Acceptor	4	3	6	4	4
No.of Hydrogen bond Donar	Nil	Nil	Nil	1	1
No.of Rule of 5 Violation	Nil	Nil	Nil	Nil	Nil
No. of rotatable bonds	5	3	3	2	2
Molar Volume	275.97	206.19	202.11	186.79	186.79

Where,

TPSA is the Topological Polar Surface Area.

From the above results all the compounds obeyed the Lipinski Rule of 5 and Rule of 7. Thus the proposed compounds which had good predictive activity and drug likeness properties were taken up for Toxicity prediction by using the OSIRIS® software.

PREDICTION OF TOXICITY:

The docked molecules were tested for toxicity by Insilico Toxicity Prediction method using Osiris Property Explorer®. This online tool predicts physico-chemical and toxicological molecular properties.

Table 5. Insilico Toxicity Prediction results

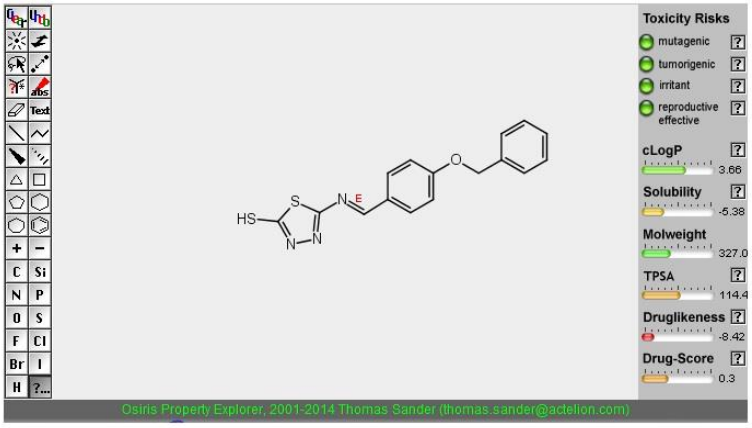
TOXICITY RISK	SAMPLE CODE				
	BOB	**CIN**	**NIB**	**OHB**	**PHB**
MUTAGENIC	Nil	Nil	Nil	Nil	Nil
TUMORIGENIC	Nil	Nil	Nil	Nil	Nil
IRRITANT	Nil	Nil	Nil	Nil	Nil
REPRODUCTIVE EFFECT	Nil	Nil	Nil	Nil	Nil

[-] – indicates absence of toxicity

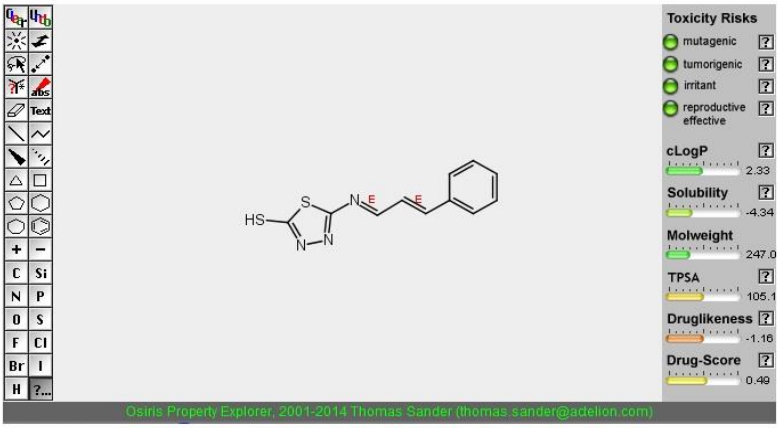
[+] – indicates presence of toxicity

Snapshot of the Insilico Toxicity Predictions:

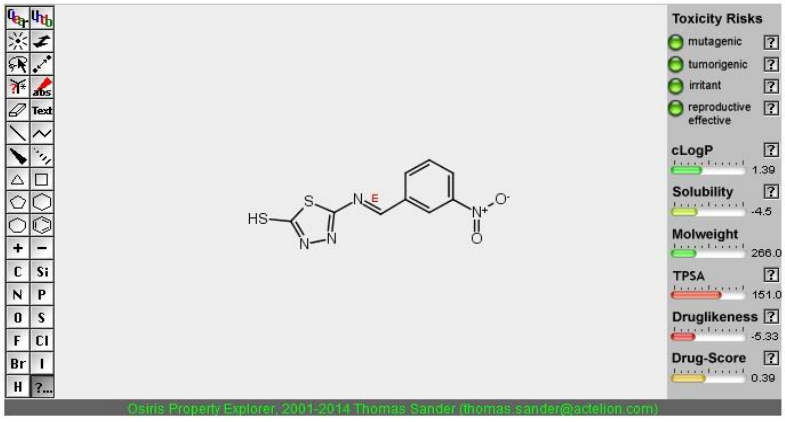
Sample: BOB



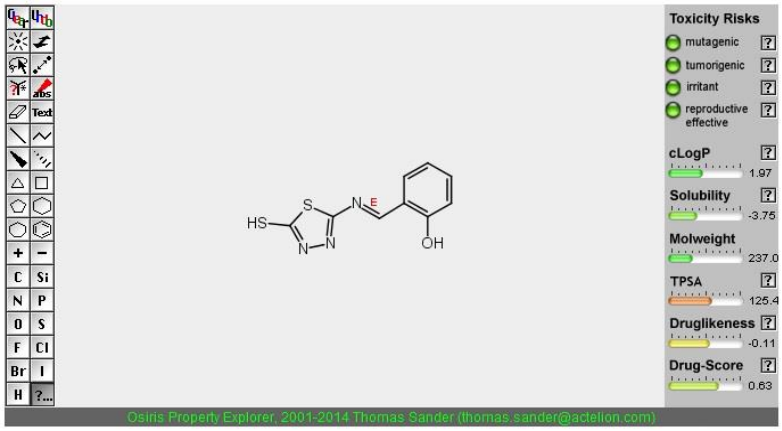
Sample: CIN



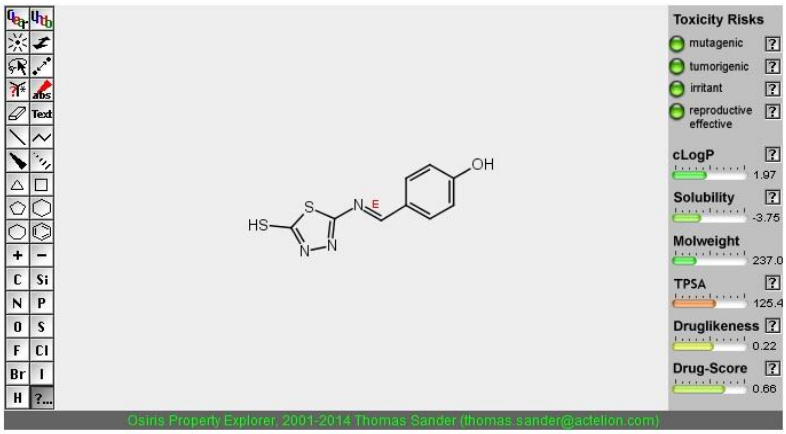
Sample: NIB



Sample: OHB



Sample: PHB



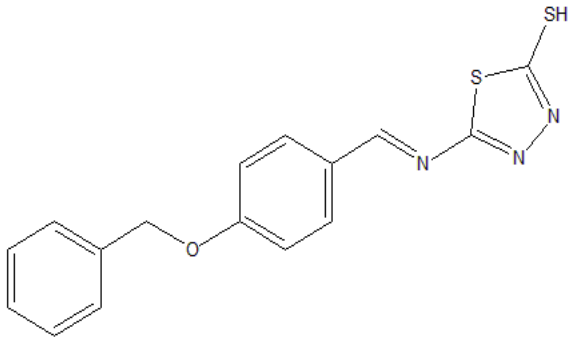
None of the compounds BOB, CIN, NIB, OHB and PHB exhibited any kind of toxicity. These compounds will be synthesis by easy way of synthetic process (Schiff base synthesis).

SYNTHETIC EFFORTS:

The synthesis was performed by the conventional method. The reaction was carried out by using 0.1 mole of 5-Amino-1,3,4-Thiadiazole-2-thiol, 0.1 mol of substituted aromatic aldehyde and ethanol acts as catalyst. The mixture was refluxed for about 4-5 hrs at 60 – 80°C. This leads to the formation of Schiff Base.

Results of the Synthetic Scheme and Characterization:

Sample Code: BOB



PROFILE:

IUPAC NAME: 5-({(*E*)-[4-(benzyloxy)phenyl]methylidene}amino)-1,3,4-thiadiazole-2-thiol

Molecular Formula	: $C_{16}H_{13}N_3OS_2$
Formula Weight	: 327.42392
Composition	: C(58.69%) H(4.00%) N(12.83%) O(4.89%) S(19.59%)
Appearance	: Pale Yellow colour powder
Solubility	: Ethanol , Methanol and Chloroform
R_f Value	: 0.56
Melting Point	: 129-131°C
Molar Refractivity	: 94.32 ± 0.5 cm^3
Molar Volume	: 248.4 ± 7.0 cm^3
Parachor	: 664.4 ± 8.0 cm^3
Index of Refraction	: 1.683 ± 0.05
Surface Tension	: 51.1 ± 7.0 dyne/cm
Density	: 1.31 ± 0.1 g/cm^3

Polarizability : 37.39 ± 0.5 $10^{-24}cm^3$

Monoisotopic Mass : 327.050002 Da

Nominal Mass : 327 Da

Average Mass : 327.4239 Da

IR Spectrum: BOB

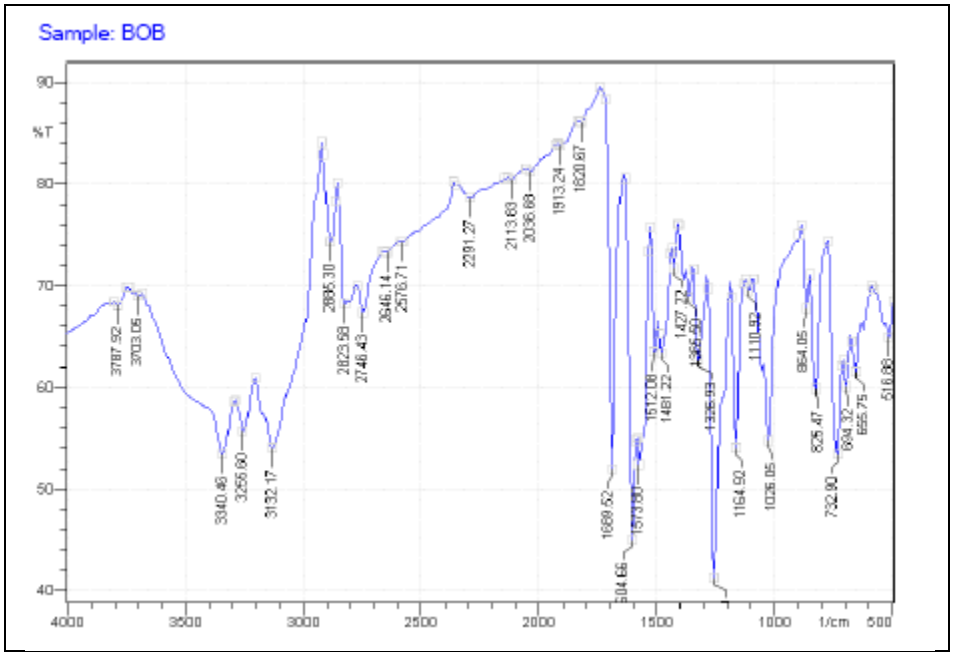
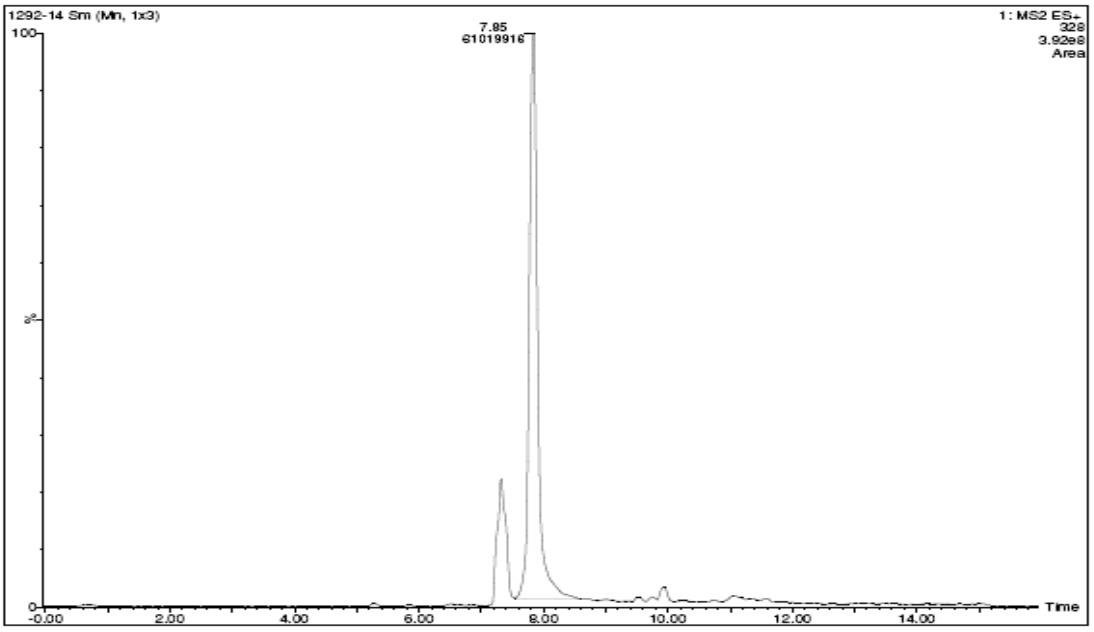


Table 6. Interpretation of IR Spectrum – BOB

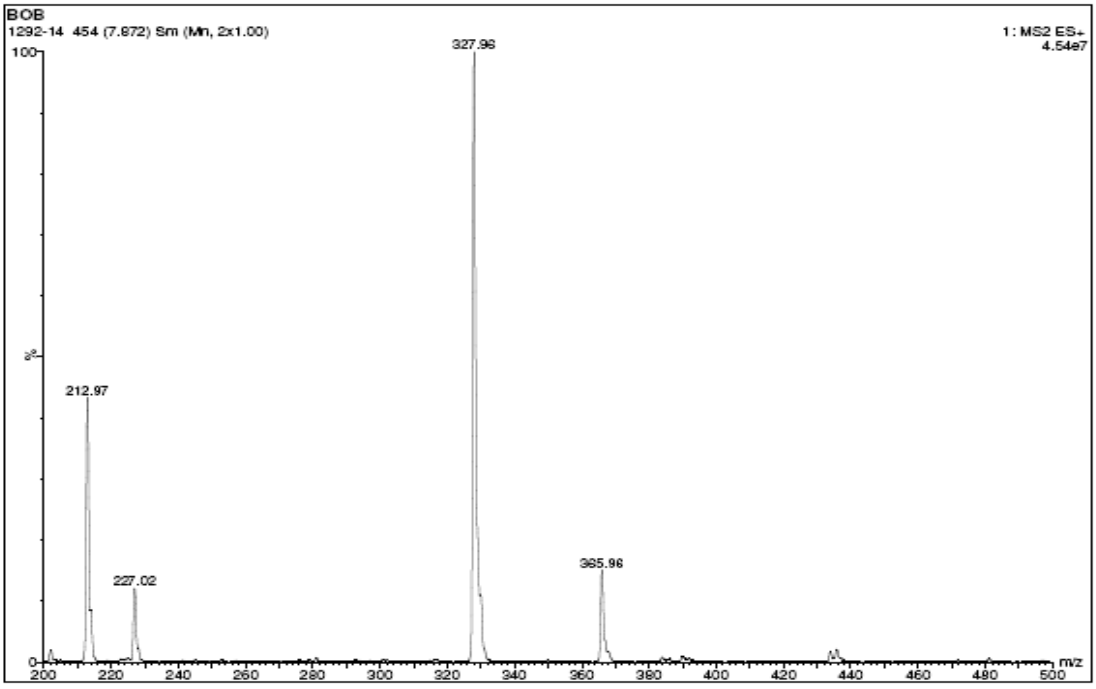
S.No.	Functional Group Region	Wave number (cm^{-1})
1	Aromatic -C-H stretching	3132.17 cm^{-1}
2	Aliphatic $–CH_2-$ stretching	2885.30 cm^{-1}
3	-C=N- stretching	1604.66 cm^{-1}
4	-C-O-C- stretching	1257.50 cm^{-1}
5	-C-S-C- stretching	694.32 cm^{-1}

LC-MS Spectrum: BOB

Chromatogram: BOB



Mass Spectrum: BOB



Calculated Mass: 327.42 g/mol

Actual Mass: 327.96 g/mol

H^1 NMR Spectrum: BOB

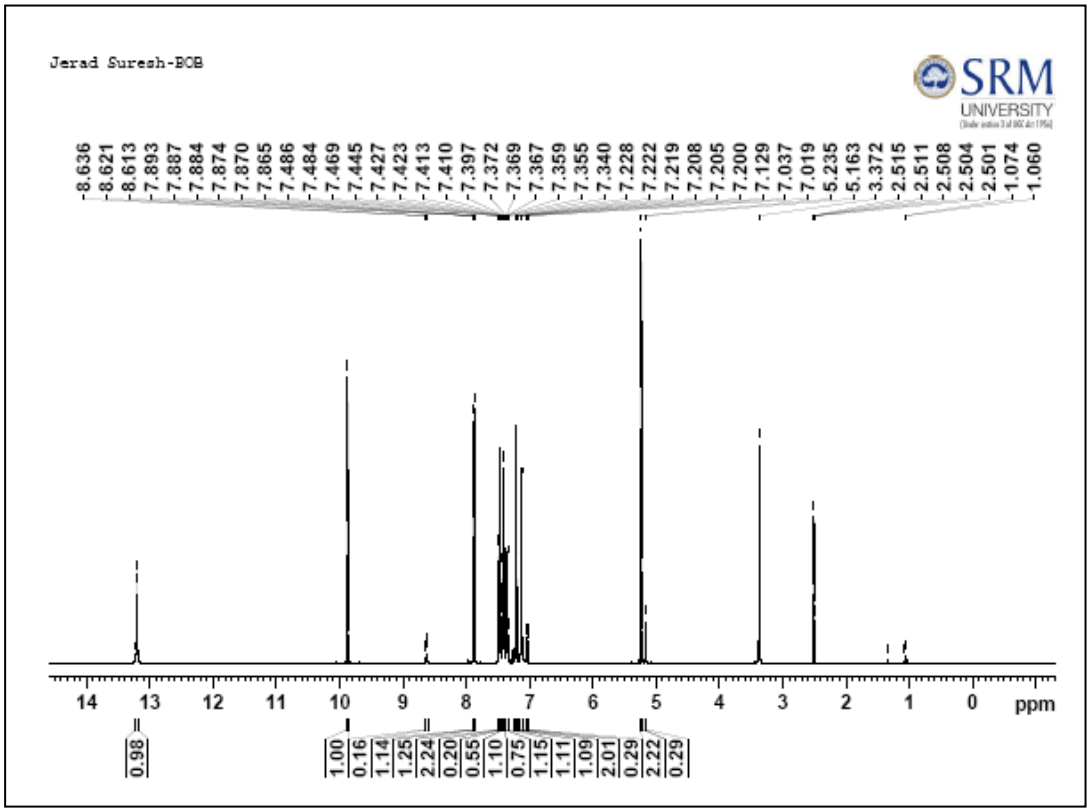
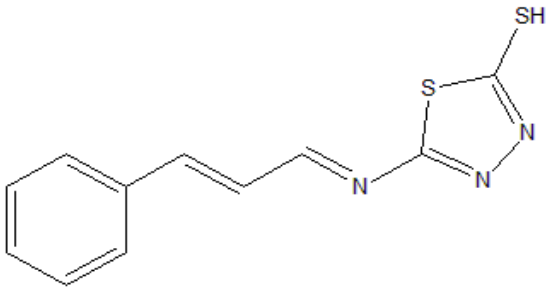


Table 7. Interpretation of H^1 NMR Spectrum – BOB

S.No.	Delta Value (δ) ppm	Nature of peak	No.of proton
2	5.3	Triplet	2
3	7.1-7.5	Multiplet	6
4	8.6-8.7	Multiplet	4
5	9.9	Singlet	1

Sample Code: CIN



IUPAC NAME: 5-{[(1*E*,2*E*)-3-phenylprop-2-en-1-ylidene]amino}-1,3,4-thiadiazole-2-thiol

Molecular Formula	: $C_{11}H_9N_3S_2$
Formula Weight	: 247.33926
Composition	: C(53.42%) H(3.67%) N(16.99%) S(25.93%)
Appearance	: Yellow colour powder
Solubility	: Ethanol, Methanol and Chloroform
R_f Value	: 0.49
Melting Point	: 115-117^oC
Molar Refractivity	: 72.44 ± 0.5 cm^3
Molar Volume	: 190.0 ± 7.0 cm^3
Parachor	: 507.0 ± 8.0 cm^3
Index of Refraction	: 1.687 ± 0.05
Surface Tension	: 50.7 ± 7.0 dyne/cm
Density	: 1.30 ± 0.1 g/cm^3
Polarizability	: 28.71 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	: 247.023787 Da
Nominal Mass	: 247 Da
Average Mass	: 247.3393 Da

IR Spectrum: CIN

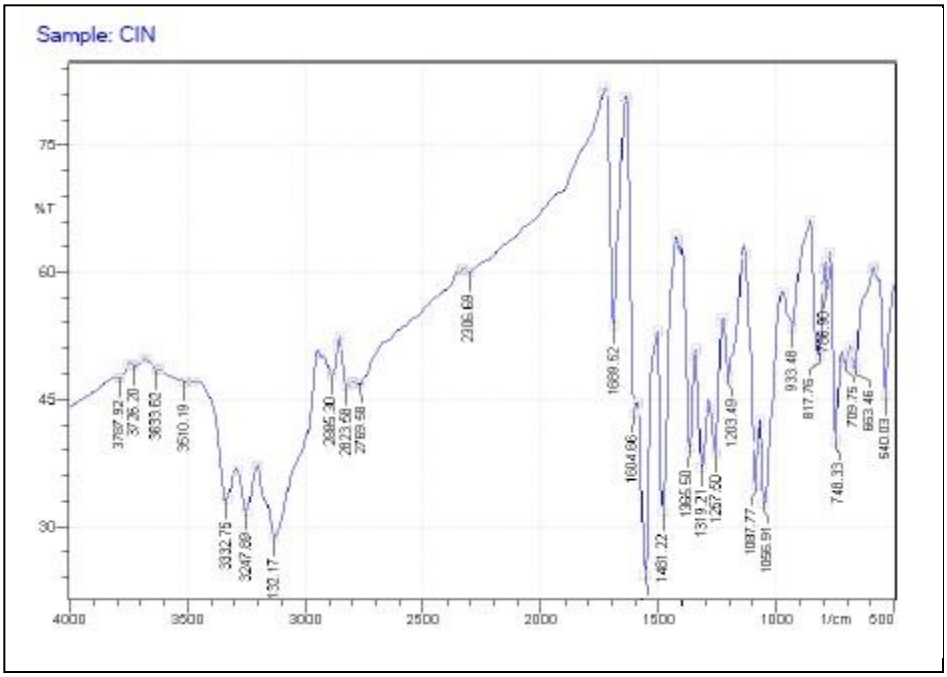
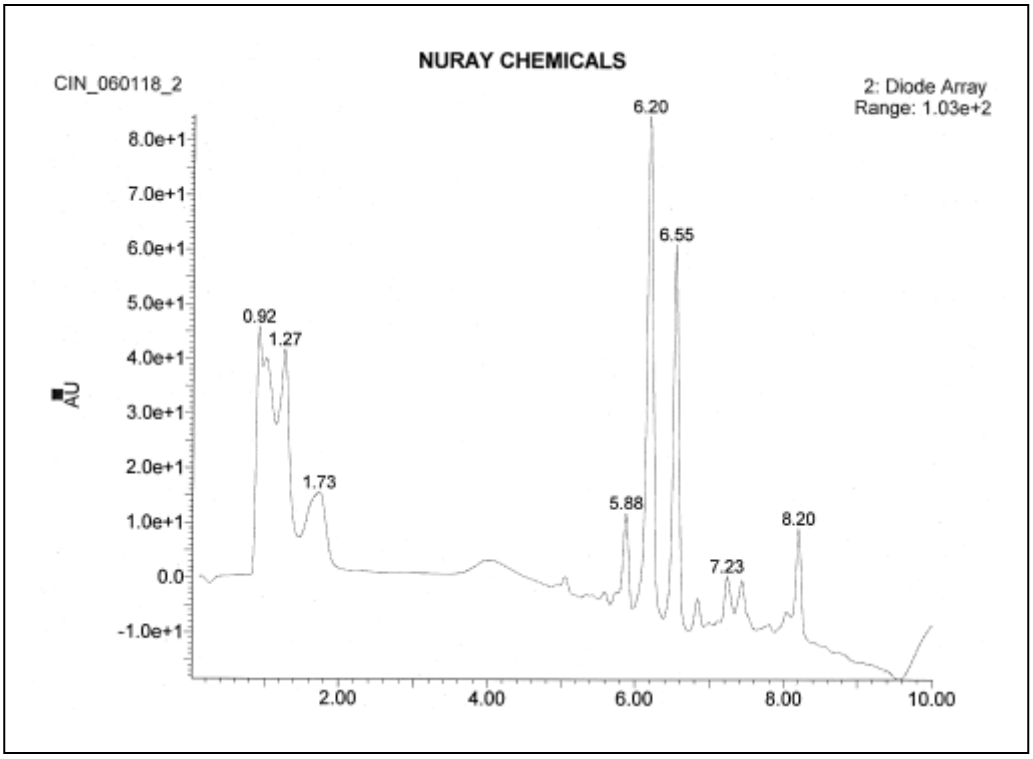


Table 8. Interpretation of IR Spectrum – CIN

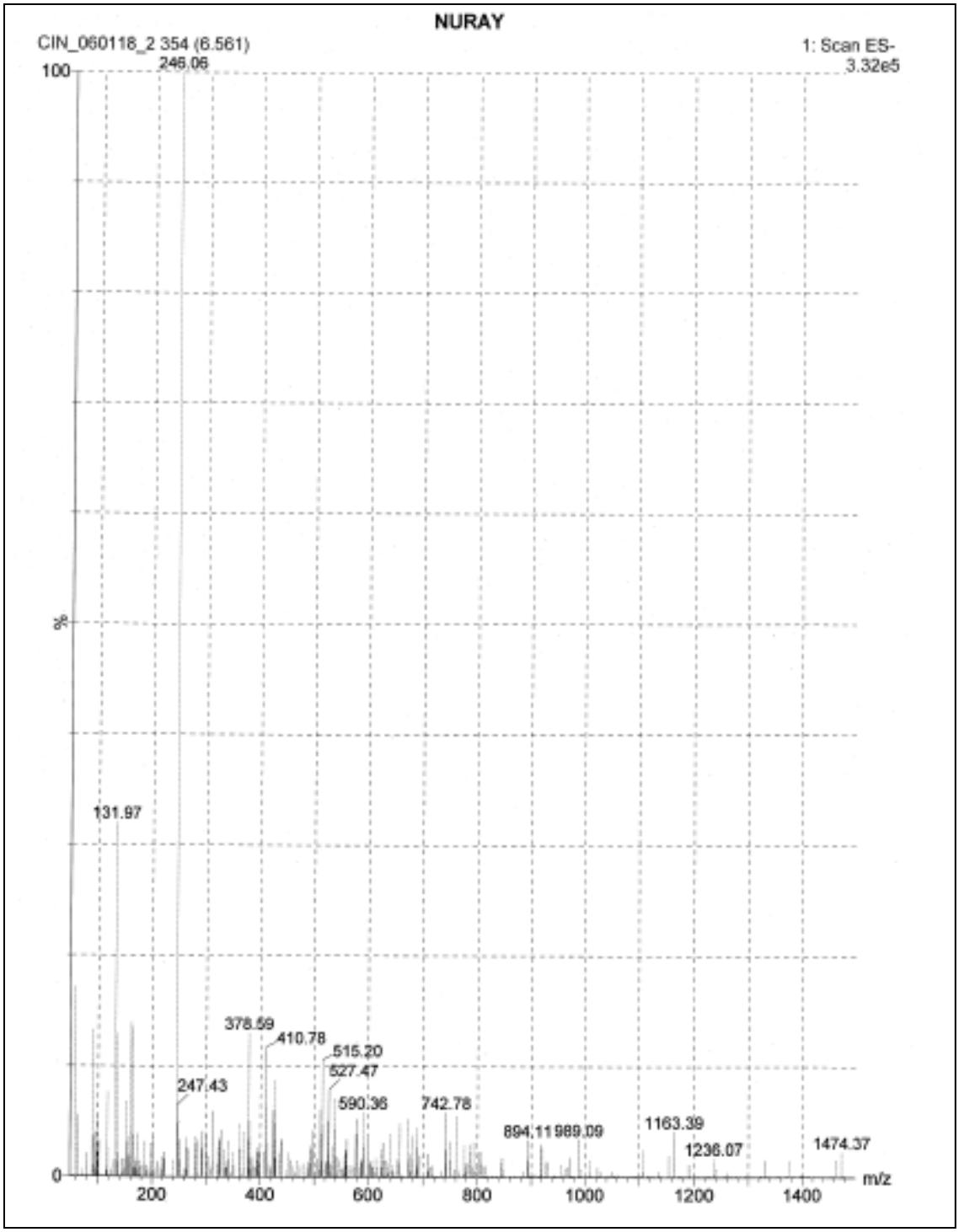
S.No.	Functional Group Region	Wave number (cm^{-1})
1	Aromatic -C-H stretching	3132.17 cm^{-1}
2	Alkene –CH=CH- stretching	1689.52 cm^{-1}
3	-C=N- stretching	1604.66 cm^{-1}
4	-C-S-C- stretching	694.32 cm^{-1}

LC-MS Spectrum: CIN

Chromatogram: CIN



Mass Spectrum: CIN



Calculated Mass: 247.33 g/mol

Actual Mass: 246.06 g/mol

H^1 NMR Spectrum: CIN

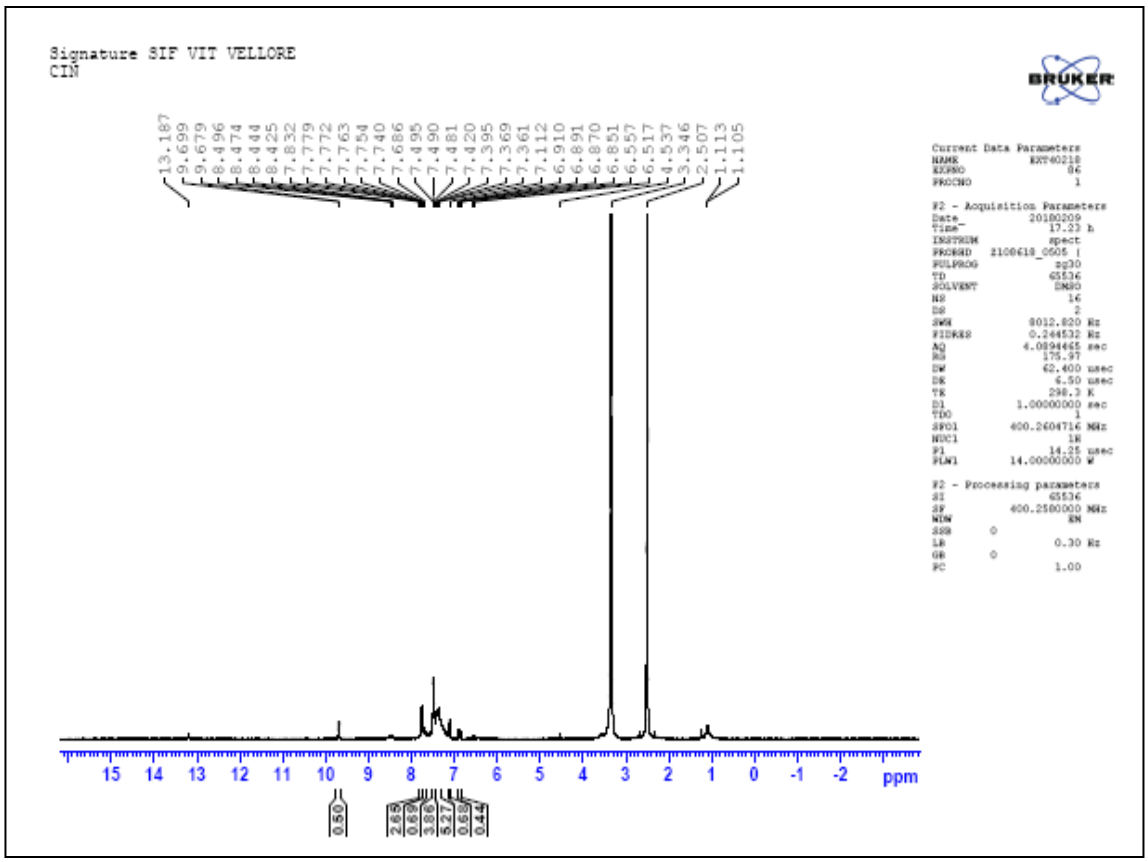
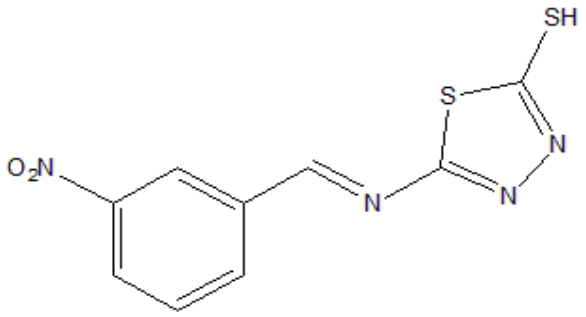


Table 9. Interpretation of H^1 NMR Spectrum – CIN

S.No.	Delta Value (δ) ppm	Nature of peak	No.of proton
1	6.8	Singlet	1
2	7.2-7.7	Multiplet	7
3	9.7	Singlet	1

Sample Code: NIB



IUPAC NAME: 5- [(*E*) - (3-nitrophenyl) methylidene] amino}-1,3,4-thiadiazole-2-thiol

Molecular Formula	: $C_9H_6N_4O_2S_2$
Formula Weight	: 266.29954
Composition	: C(40.59%) H(2.27%) N(21.04%) O(12.02%) S(24.08%)
Appearance	: Yellowish Green colour Powder
Solubility	: Ethanol, Methanol and Chloroform
R_f Value	: 0.53
Melting Point	: 170-171ºC
Molar Refractivity	: 68.88 ± 0.5 cm^3
Molar Volume	:163.2 ± 7.0 cm^3
Parachor	: 475.2 ± 8.0 cm^3
Index of Refraction	: 1.786 ± 0.05
Surface Tension	: 71.9 ± 7.0 dyne/cm
Density	: 1.63 ± 0.1 g/cm^3
Polarizability	: 27.30 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	: 265.993215 Da
Nominal Mass	: 266 Da
Average Mass	: 266.2995 Da

IR Spectrum: NIB

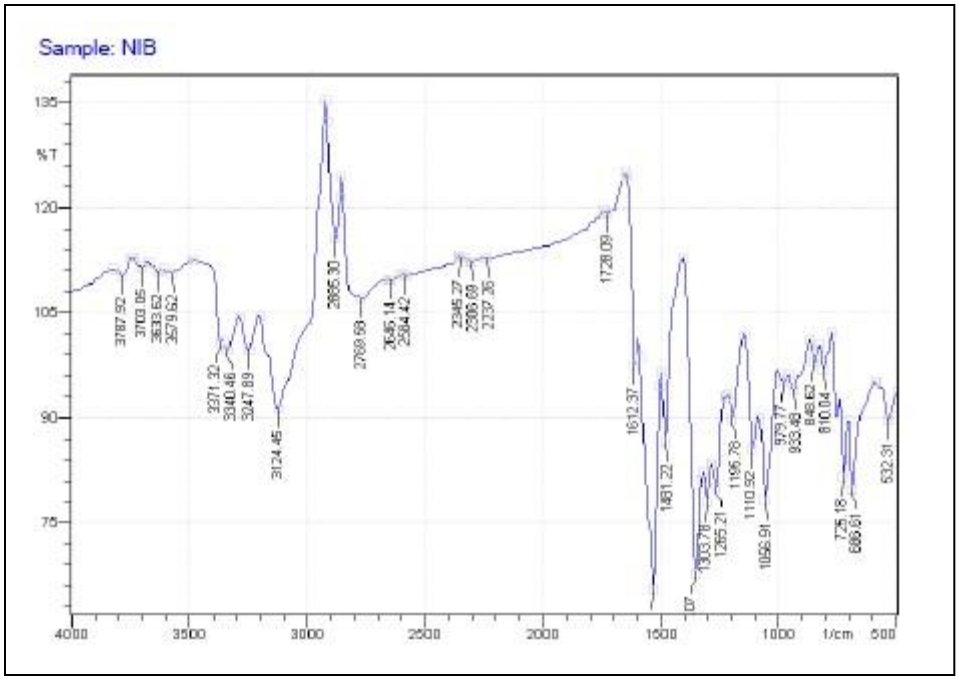
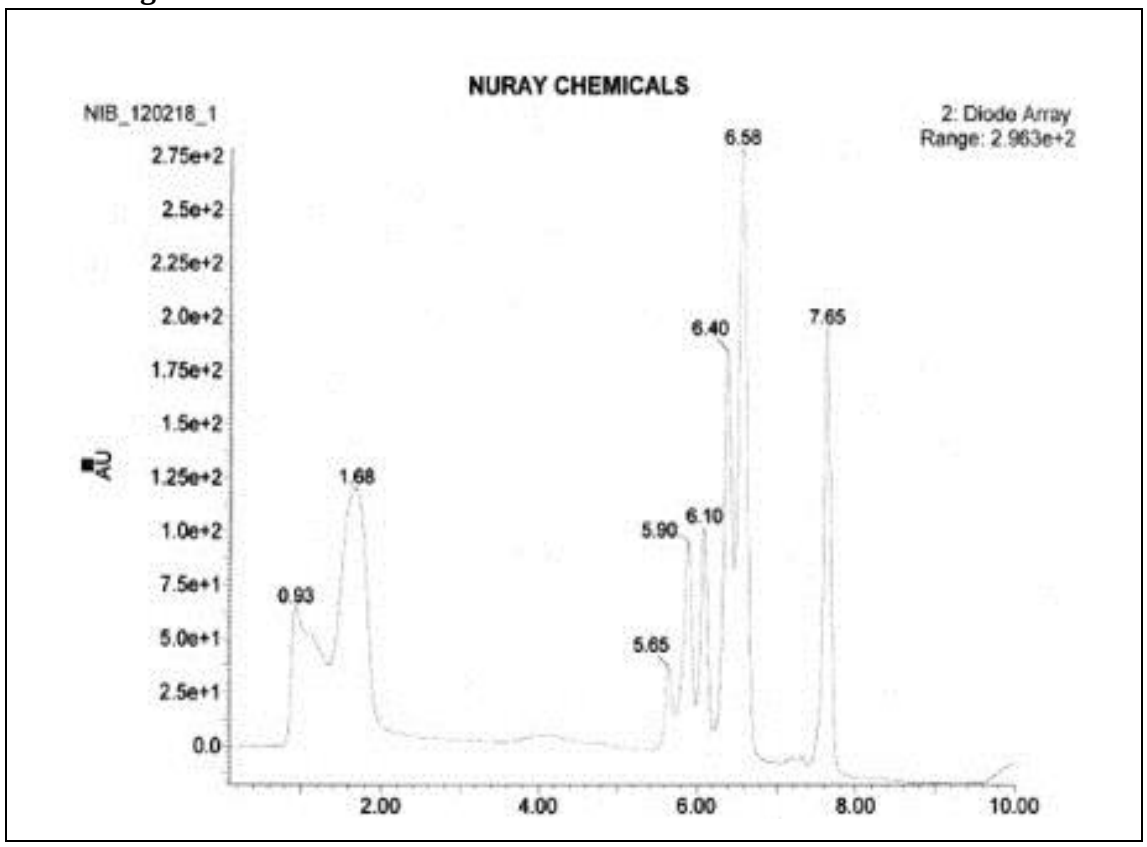


Table 10. Interpretation of IR Spectrum – NIB

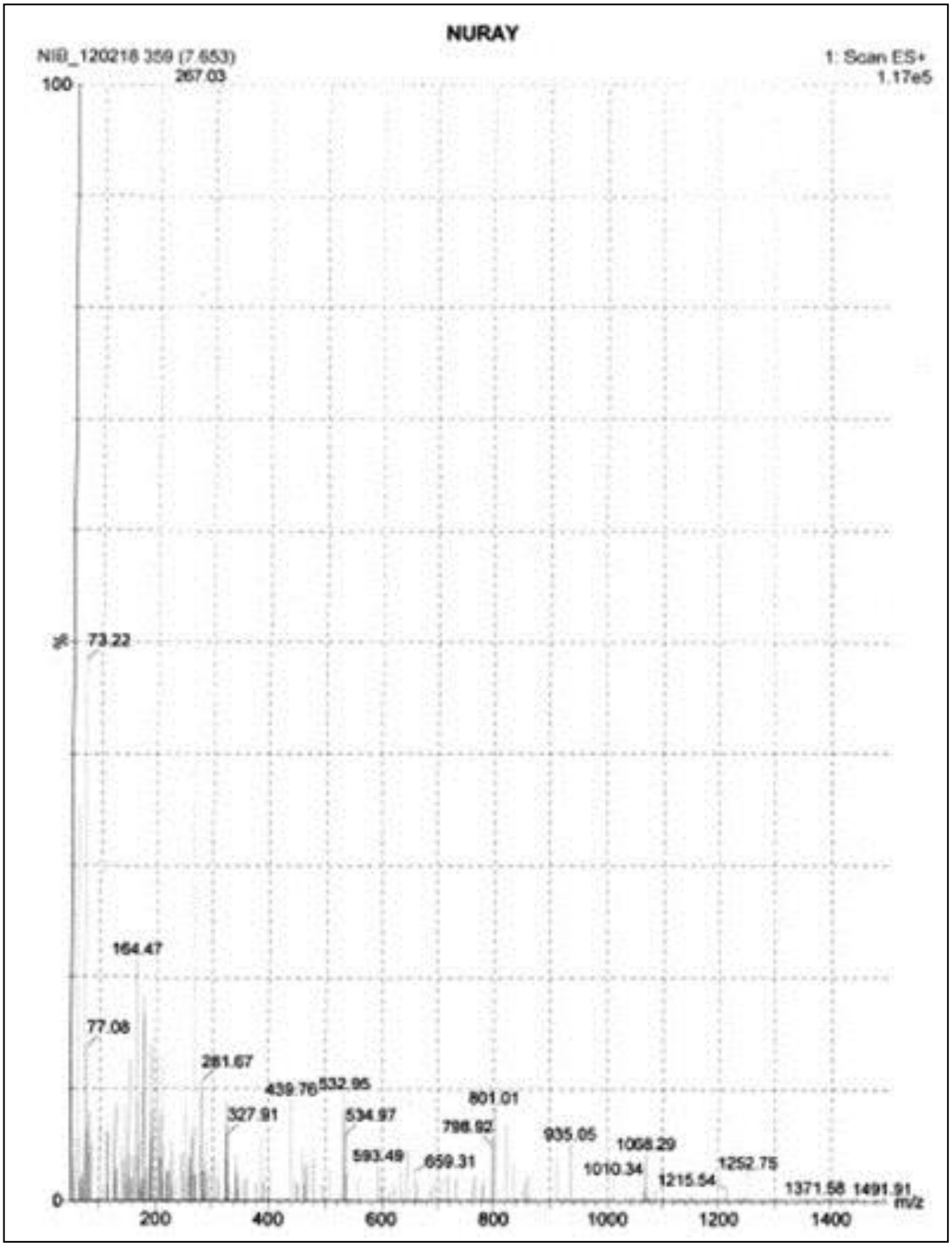
S.No.	Functional Group Region	Wave number (cm^{-1})
1	Aromatic –C-H- stretching	3124.45 cm
2	-C=N- stretching	1612.37 cm
3	-C-NO_2 stretching	1481.22 cm
4	-C-S-C- stretching	686.61 cm

LC-MS Spectrum: NIB

Chromatogram: NIB



Mass Spectrum: NIB



Calculated Mass: 266.29 g/mol

Actual Mass: 267.03 g/mol

H^1 NMR Spectrum: NIB

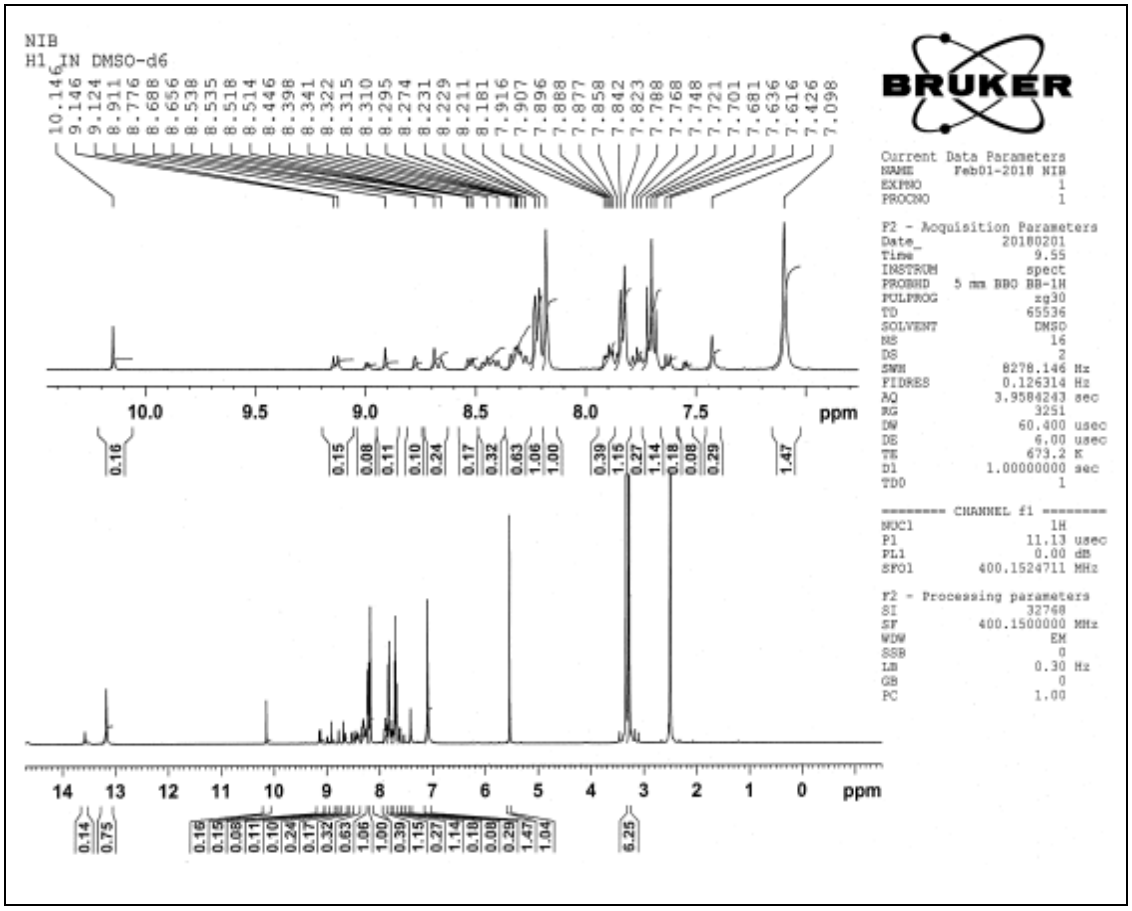
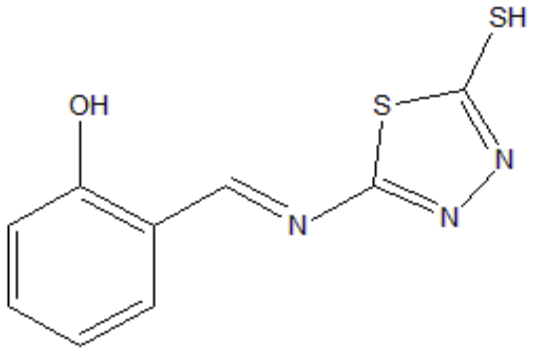


Table 11. Interpretation of H^1 NMR Spectrum – NIB

S.No.	Delta Value (δ) ppm	Nature of peak	No.of proton
1	5.5	Doublet	1
2	7.1-8.3	Multiplet	4
3	13.2	Singlet	1

Sample Code: OHB



IUPAC NAMES : 2-{(*E*)-[(5-sulfanyl- 1,3,4-thiadiazol-2-yl) imino] methyl}phenol

Molecular Formula	: $C_9H_7N_3OS_2$
Formula Weight	: 237.30138
Composition	: C(45.55%) H(2.97%) N(17.71%) O(6.74%) S(27.02%)
Appearance	: Paale Yellow colour powder
Solubility	: Ethanol, Methanol and Chloroform
R_f Value	: 0.44
Melting Point	: 146-148^oC
Molar Refractivity	**:** 64.07 ± 0.5 cm^3
Molar Volume	: 155.0 ± 7.0 cm^3
Parachor	: 435.4 ± 8.0 cm^3
Index of Refraction	: 1.764 ± 0.05
Surface Tension	: 62.1 ± 7.0 dyne/cm
Density	: 1.53 ± 0.1 g/cm^3
Polarizability	: 25.40 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	: 237.003052 Da
Nominal Mass	: 237 Da
Average Mass	: 237.3014 Da

IR Spectrum: OHB

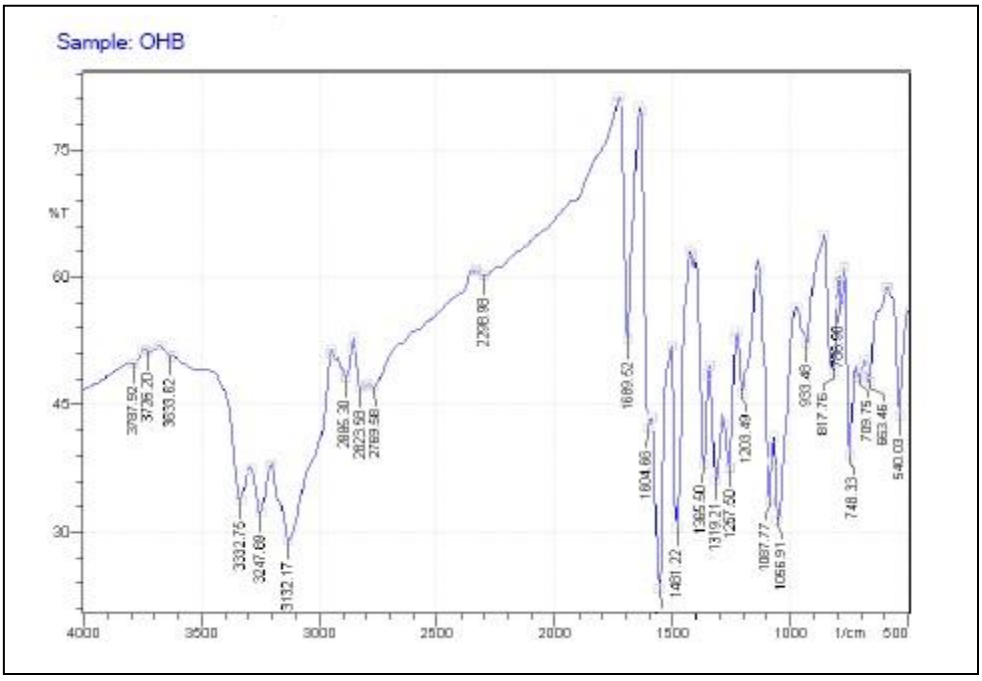
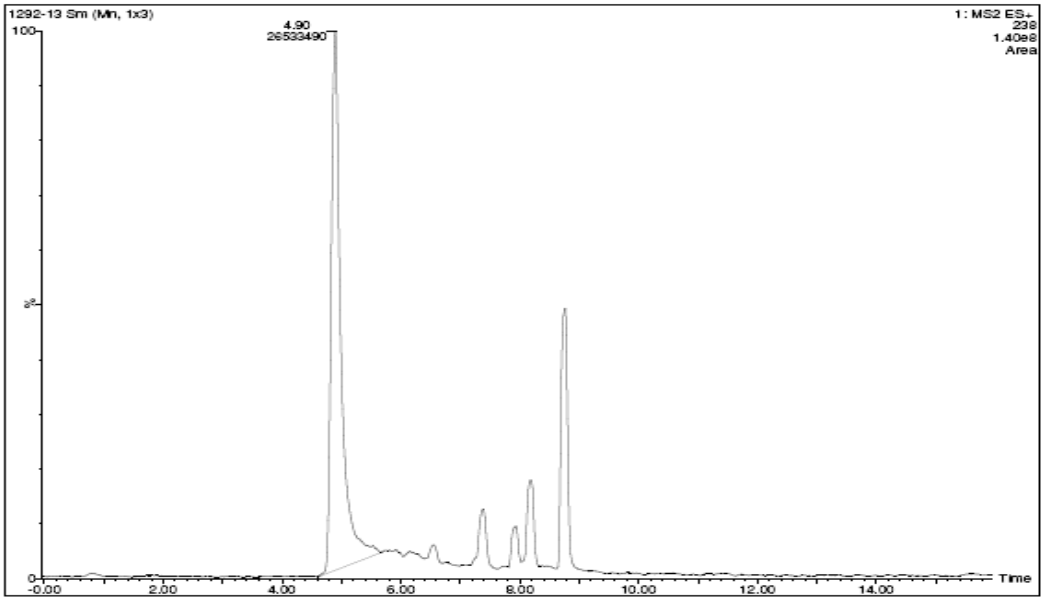


Table 12. Interpretation of IR Spectrum - OHB

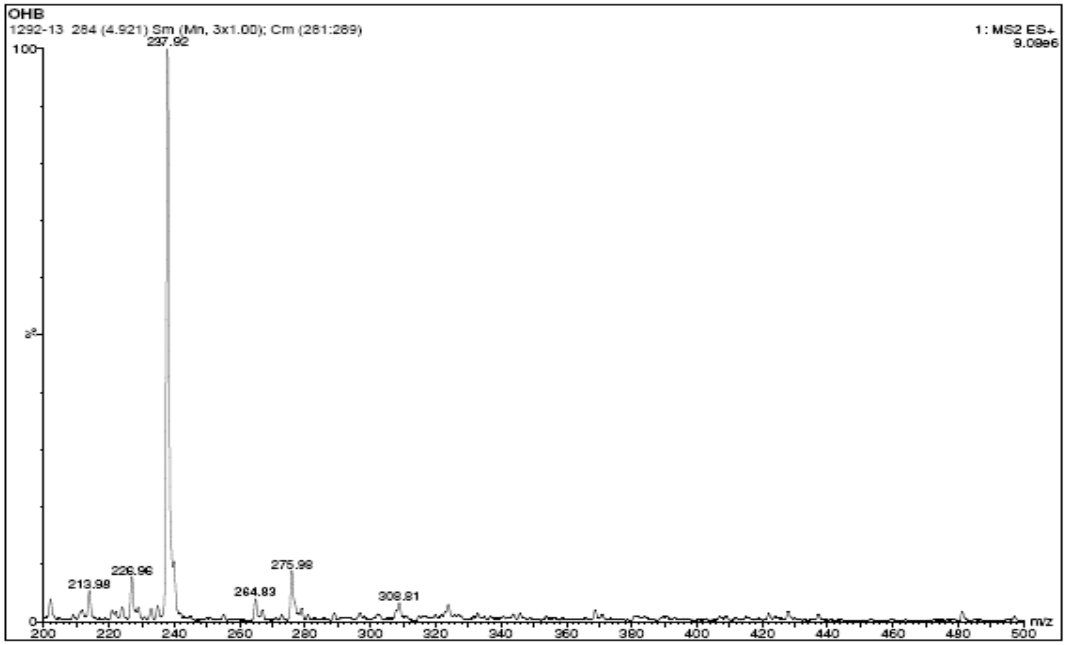
S.No.	Functional Group Region	Wave number (cm^{-1})
1	Alcoholic –OH stretching	3633.62 cm^{-1}
2	Aromatic –C-H- stretching	3132.17 cm^{-1}
3	-C=N- stretching	1604.66 cm^{-1}
4	-C-O- stretching	1280.64 cm^{-1}
5	-C-S-C- stretching	694.32 cm^{-1}

LC-MS Spectrum: PHB

Chromatogram: PHB



Mass Spectrum: PHB



Calculated Mass: 237.30 g/mol

Actual Mass: 237.92 g/mol

H^1 NMR Spectrum: OHB

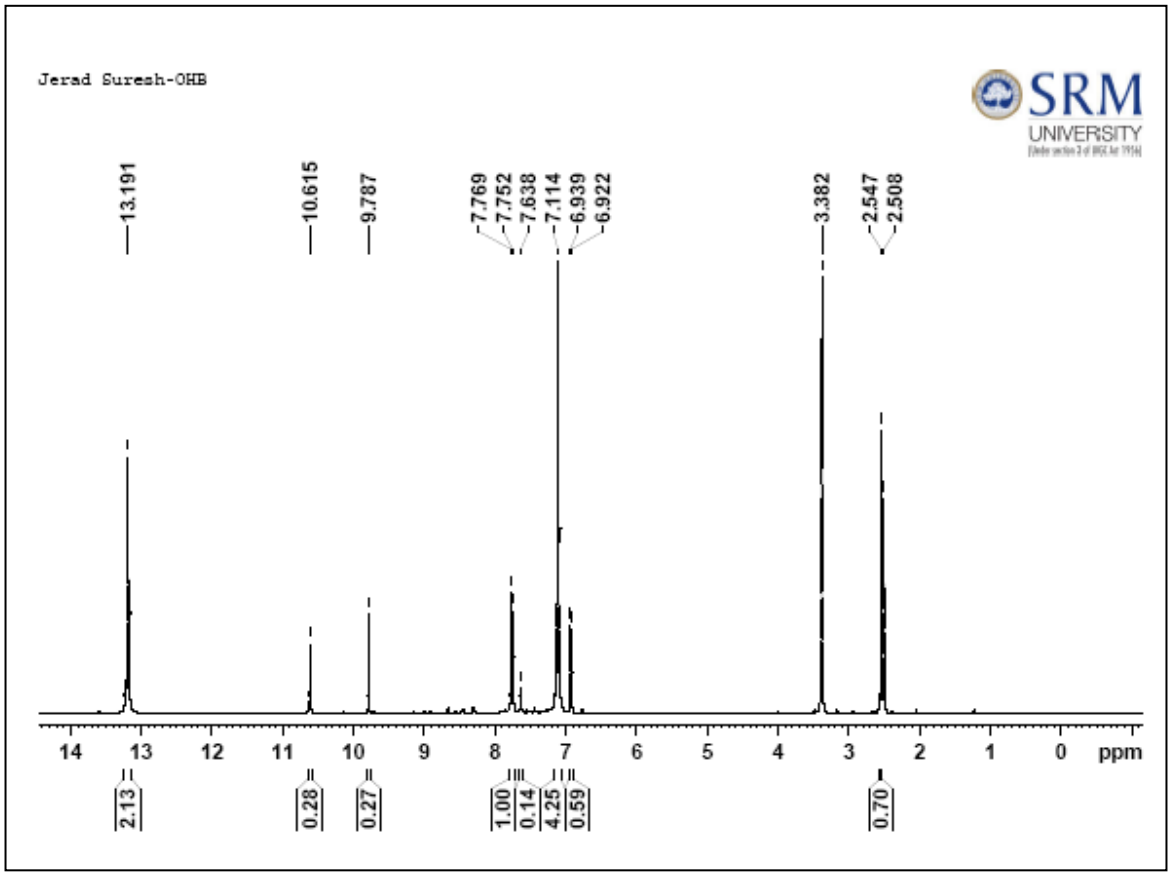
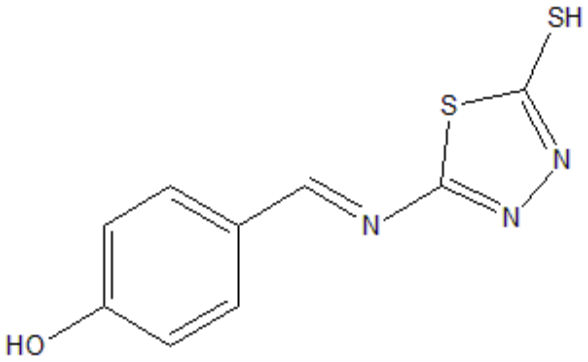


Table 13. Interpretation of H^1 NMR Spectrum – OHB

S.No.	Delta Value (δ) ppm	Nature of peak	No.of proton
1	7.1-7.2	Multiplet	4
2	7.8	Singlet	1
3	13.2	Triplet	2

Sample Code: PHB



IUPAC NAMES: 4 - {(*E*) - [(5-sulfanyl- 1,3,4- thiadiazol-2-yl) imino]methyl}phenol

Molecular Formula	: $C_9H_7N_3OS_2$
Formula Weight	: 237.30138
Composition	: C(45.55%) H(2.97%) N(17.71%) O(6.74%) S(27.02%)
Appearance	: Pale Brown colour powder
Solubility	: Ethanol, Methanol and Chloroform
R_f Value	: 0.46
Melting	: 174-176^oC
Molar Refractivity	: 64.07 ± 0.5 cm^3
Molar Volume	: 155.0 ± 7.0 cm^3
Parachor	: 435.4 ± 8.0 cm^3
Index of Refraction	: 1.764 ± 0.05
Surface Tension	: 62.1 ± 7.0 dyne/cm
Density	: 1.53 ± 0.1 g/cm^3
Polarizability	: 25.40 ± 0.5 $10^{-24}cm^3$
Monoisotopic Mass	: 237.003052 Da
Nominal Mass	: 237 Da
Average Mass	: 237.3014 Da

IR Spectrum: PHB

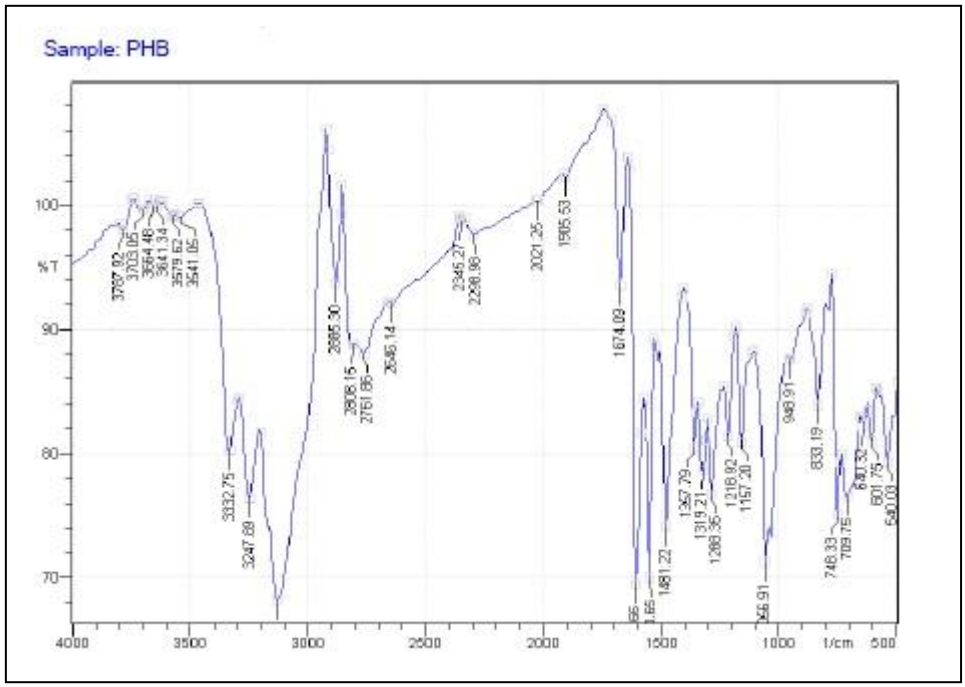
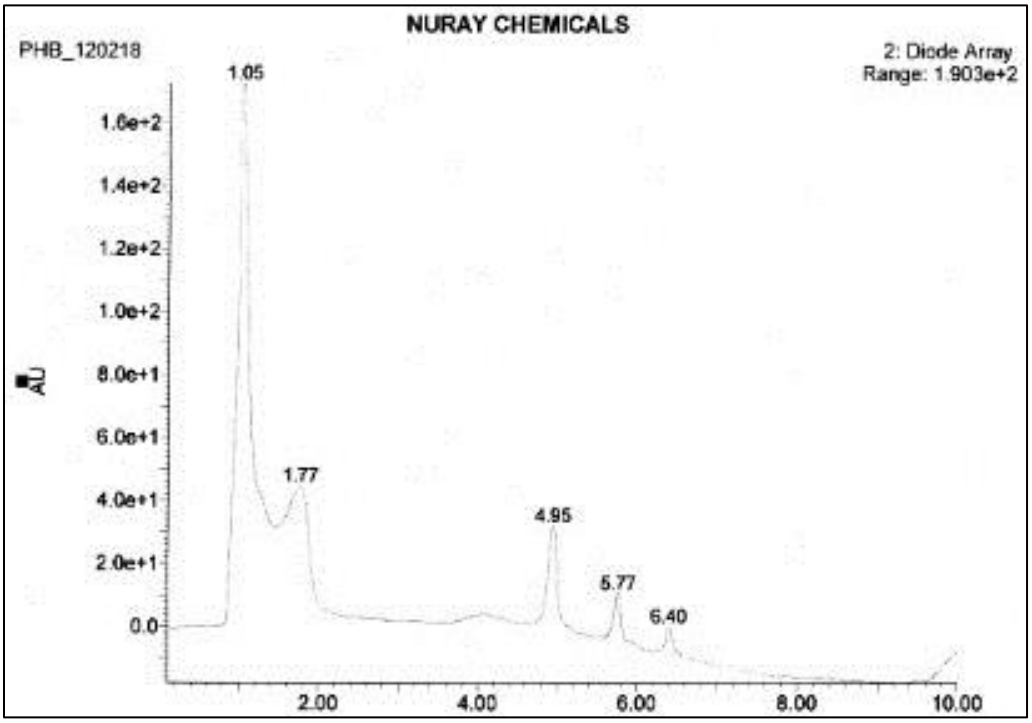


Table 14. Interpretation of IR Spectrum – PHB

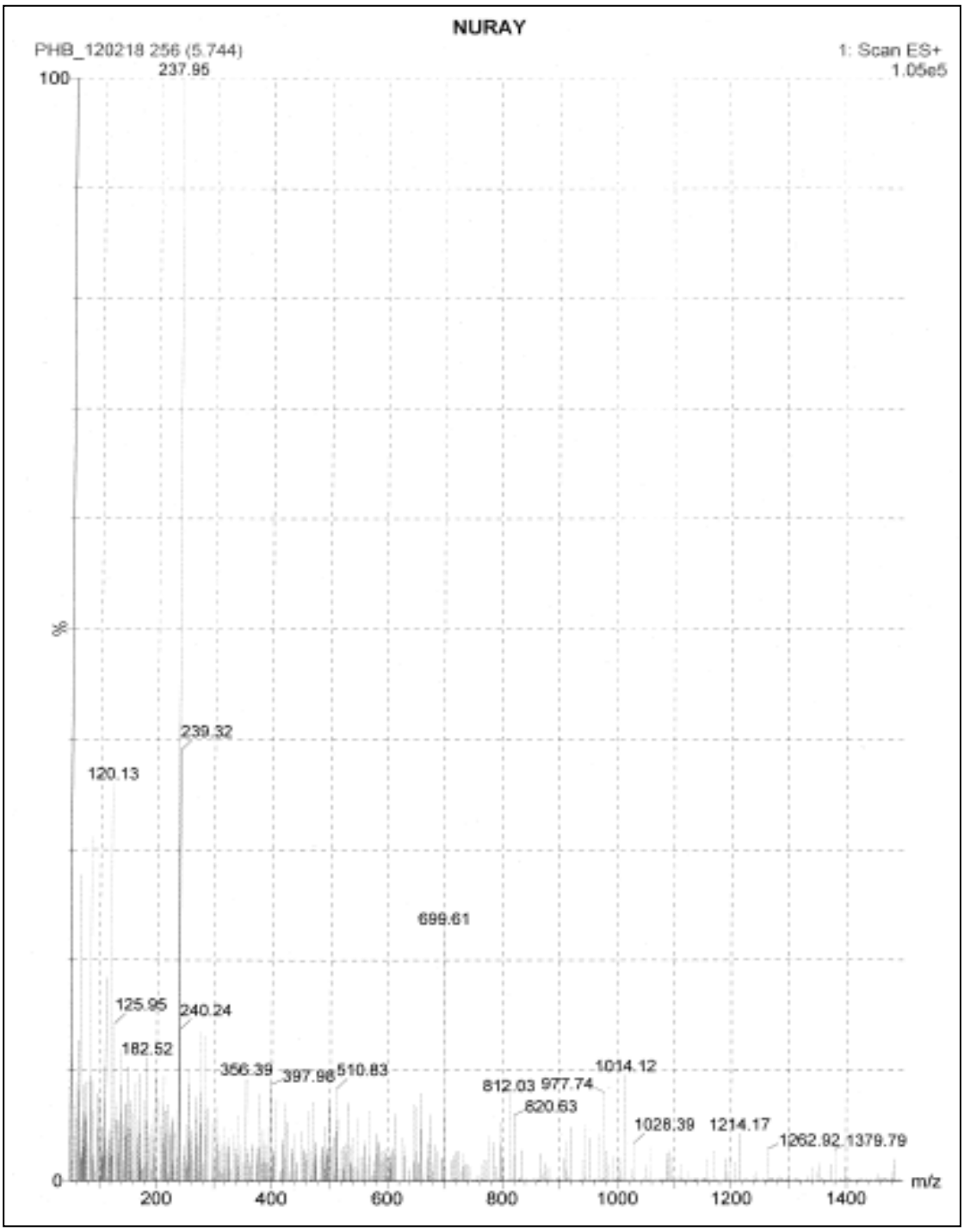
S.No.	Functional Group Region	Wave number (cm^{-1})
1	Alcoholic –OH	3625.91 cm^{-1}
2	Aromatic –C-H- stretching	3132.27 cm^{-1}
3	-C=N- stretching	1604.66 cm^{-1}
4	-C-O- stretching	1288.35 cm^{-1}
5	-C-S-C-	709.75 cm^{-1}

LC-MS Spectrum: PHB

Chromatogram: PHB



Mass Spectrum: PHB



Calculated Mass: 237.30 g/mol

Actual Mass: 237.95 g/mol

H^1 NMR Spectrum: PHB

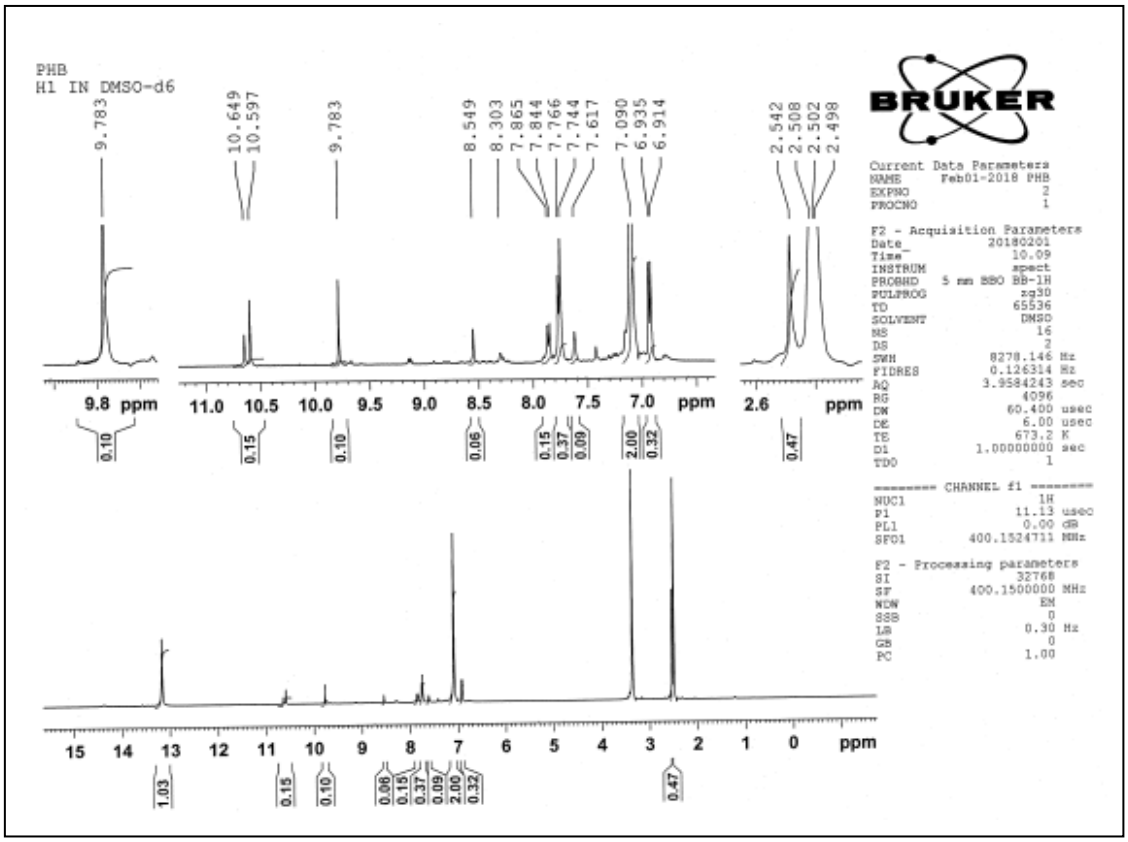


Table 15. Interpretation of H^1 NMR Spectrum – PHB

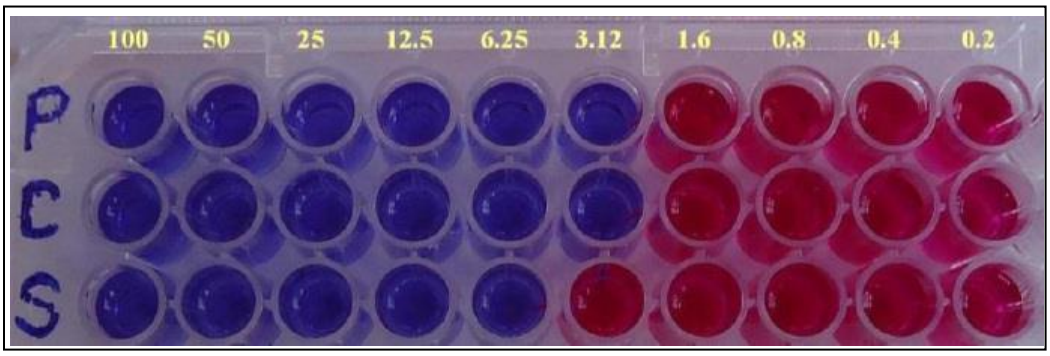
S.No.	Delta Value (δ) ppm	Nature of peak	No.of proton
1	6.9	Singlet	1
2	7.1-7.2	Multiplet	4
3	13.2	Singlet	1

BIOLOGICAL EVALUATION:

MABA REPORT OF THE SYNTHESISED COMPOUNDS:

All the synthesized compounds were tested against ***Mycobacterium tuberculosis*** (Vaccine strain, H37Rv strain): ATCC No- 27294. The compounds showed anti-mycobacterial activity in a varying degree against the organism tested. The organism tested was susceptible to all the synthesized compounds and the minimum inhibitory concentration for the compounds varied between 12.5 and 3.125 mcg/ml. The data pertaining to these observations are presented in the table. Inhibition was compared with standard values.

Standard values for the Anti-Tb test which was performed.



Pyrazinamide- 3.125µg/ml

Ciprofloxacin-3.125µg/ml

Streptomycin- 6.25µg/ml

MIC Values for synthesized compounds

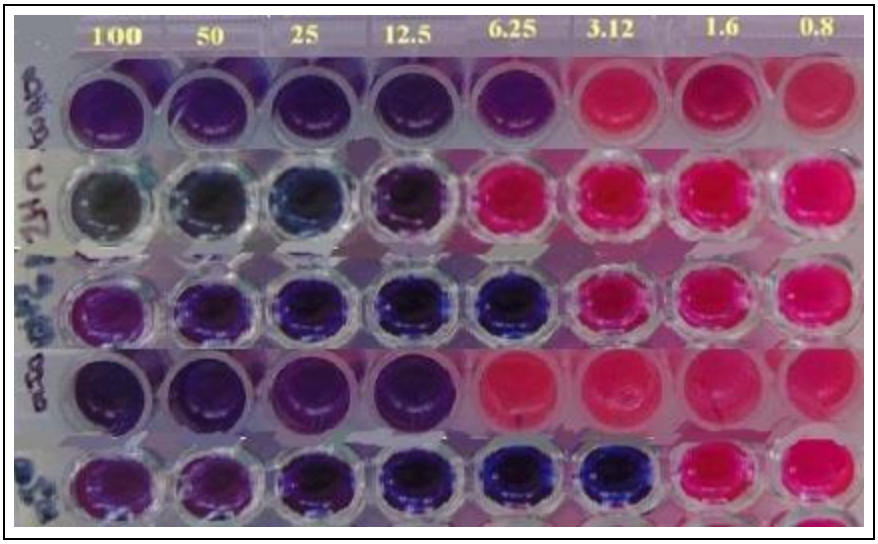


Table 16. MIC values of Synthesized compounds by MABA

Sample Code	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml	1.6 µg/ml	0.8 µg/ml
BOB	S	S	S	S	S	R	R	R
CIN	S	S	S	S	R	R	R	R
NIB	S	S	S	S	S	R	R	R
OHB	S	S	S	S	R	R	R	R
PHB	S	S	S	S	S	S	R	R

S - Sensitive

R- Resistance

From the results, all the synthesized molecules were compared with standard drugs such as Pyrazinamide- 3.125µg/ml, Ciprofloxacin-3.125µg/ml, Streptomycin-6.25µg/ml. Among them molecule PHB showed better MIC value of 3.125 µg/ml, and molecule BOB and NIB showed MIC values of 6.25 µg/ml. As comparing with standard drugs, the synthesized comounds PHB, BOB and NIB is more active. From the results only 4 compounds were selected for Acute Toxicity Study.

ACUTE ORAL TOXICITY STUDY:

The active compounds from the MABA report BOB, NIB, OHB, PHB were chosen for acute oral toxicity study using albino mice (OECD guidelines(423)). After administration of molecules animals were observed for behavioral signs of toxicity like motor activity, tremor etc., and no significant toxic signs were observed during 14 days which was tabulated below.

Table 17. Acute Oral Toxicity study

S.No.	PARAMETERS	RESULTS
1.	Toxic signs	Absent
2.	Pre-terminal deaths	Nil
3.	Body weight	No specific change
4.	Motor activity	Normal
5.	Tremors	Absent
6.	Convulsions	Absent
7.	Straub reaction	Absent
8.	Righting reflex	Present
9.	Lacrimation and Salivation	Normal
10.	Unusual vocalization	Absent
11.	Sedation	Absent
12.	Body temperature	Normal
13.	Analgesia	Absent
14.	Ptosis	Absent
15.	Diarrhoea	Absent
16.	Skin colour	Normal
17.	Respiration	Normal
18.	Scratching	Absent
19.	Aggressiveness and restlessness	Absent

The results of the acute toxicological studies revealed that the administration of 4 molecules by oral route upto 2000mg/kg/b.w did not produce any mortality and it was tolerated.

CYTOTOXICITY EVALUATION:

The more active drugs BOB, NIB, OHB, PHB were chosen for Cytotoxicity study on Vero cell line and the results of Inhibitory concentration were tabulated below.

Table 18. Cytotoxicity results

Concentration (µg/ml)	**BOB**	**NIB**	**OHB**	**PHB**
500	98.06	69.74	49.27	53.54
250	72.52	57.38	35.84	31.23
125	48.85	39.96	19.35	22.06
64.5	26.06	20.83	7.78	9.65
31.25	15.13	10.03	0.34	4.08
IC_{50} from Prism	**121.5**	**201.9**	**472.9**	**456.2**

IC_{50} – Half maximal inhibitory concentration

The IC_{50} for Rifampicin is 113 µg/ml on vero cell line. The reported values of the compounds were compared with standard drugs.

Therefore as compared to Rifampicin the synthesized compounds were found to be more cytotoxic.

DISCUSSION

From literature review the thiadiazole derivatives shows Anti-tubercular activity. The synthesized molecule BOB, CIN, NIB, OHB, PHB possesses thiadiazole nucles. According to the results of this work presence of thiol (-SH) group on 2^{nd} position of 1,3,4 thiadiazole nucleus brings active compounds. In thiadiazole nucleus amino group in 5^{th} position subjected for Schiff base formation with 5 aromatic aldehydes.

Different aromatic aldehyde derivatives with thiadiazole nucleus shows different activity. Para substitution of hydroxyl (-OH) group in benzaldehyde shows better activity. Nitro substitution on 3^{rd} position and Phenyl methoxy substitution in 4^{th} position shows optimal activity. But Cinnamaldehyde shows less activity.

SUMMARY

AND

CONCLUSION

SUMMARY

- ✓ *Glutamine synthetase* I is a vital enzyme present in the cell wall of *Mycobacterium tuberculosis* $H_{37}Rv$. It belongs to the Ligase family.
- ✓ A database of 200 molecules with high prospects of inhibiting the target *Glutamine synthetase* I were carefully chosen by making changes to the known hit molecules, here the thiadiazole nucleus was chosen.
- ✓ The designed molecules were docked against the target chosen using AutoDock 4®.
- ✓ Five molecules with good docking score [lower binding energy] and interactions were shortlisted for synthesis.
- ✓ The selected molecules were subjected to toxicity prediction assessment by OSIRIS® property explorer developed by Acetilon Pharmaceuticals limited which is available online. The results are color coded as green color which predicts the drug likeness and possibly better activity.
- ✓ The reaction condition were optimized, synthesized and labelled as BOB, CIN, NIB, OHB, PHB.
- ✓ The characterization of the synthesized compounds was done using TLC, Melting point Infra-red, Mass spectrometric methods [LC-MS] and Nuclear Magnetic Resonance [H1 NMR] spectroscopy methods.
- ✓ All the Synthesized compounds exhibited molecular ion peak (M^+) of varying intensities.
- ✓ The final pure compounds were screened for Anti-mycobacterial activity by in vitro method called Microplate Alamar Blue Assay [MABA].
- ✓ The synthesized compounds showed sensitivity [Minimum inhibitory concentration] at 3.125mcg/ml. The standard drugs Pyrazinamide, Ciprofloxacin and Streptomycin exhibited anti-mycobacterial activity at 3.125 mcg/ml, 3.125 mcg/ml and 6.25 mcg/ml concentrations respectively. This indicates that the synthesized compounds are as Potent as the standard drugs.
- ✓ Based on the MABA report, Acute Oral Toxicity study were performed and observed that the administration of the synthesized molecules by oral route upto 2000mg/kg/b.w is safe.
- ✓ The selected compounds showed IC_{50} Values of 121.5, 201.9, 472.9, 456.2 µg/ml respectively for BOB, NIB, OHB, PHB. Rifampicin showed IC_{50} value of 113 µg/ml. As compared with standard drug the synthesized molecules were found to be more cytotoxic.

CONCLUSION

It is concluded that the synthesized compounds might effectively inhibit the chosen target *Glutamine Synthetase* I which is essential for the *Mycobacterial tuberculosis*. Further structural modifications of the synthesized compounds will aid in the development of potential molecule against the pathogen.

BIBLIOGRAPHY

BIBLIOGRAPHY

1. Amit Kishor Srivastava, Pharmacology: A Book Of Achieving Knowledge For Drugs, Educreation Publishing, 2017: 45.
2. Jerad Suresh, S. Geetha Lakshmi, K. Madhesh, B. Karunya, K. M. Noorulla and P. R. Surya, Design, Synthesis, Characterization And Biological Evaluation Of Novel Anti-Tubercular Agents Targeting Glutamine Synthetase-1 And Cyclopropanemycolic Acid Synthase-2, International Journal of Pharmaceutical Sciences and Research, 2018; Vol. 9(2): 563-574.
3. https://fr.123rf.com/photo_66213934_stock-illustration-bacteria-which-cause-tuberculosis-mycobacterium-tuberculosis-3d-illustration.html
4. Priyadarshi Sharma, Nidhi Sharma, Study of Demographic Status of Tuberculosis Patient, IOSR Journal of Dental and Medical Sciences, 2015; Vol. 14(8): 58-61.
5. Barberis. I, Bragazzi. N.L, Galluzzo. L, and Martini. M, The history of tuberculosis: from the first historical records to the isolation of Koch's bacillus, Journal of Preventive Medicine and Hygiene, 2017; Vol. 58(1): E9-E12.
6. Nicole Fogel, Tuberculosis: A disease without boundaries, Elsevier Ltd., 2015; 95: 527-531.
7. Bedaquiline: A new drug approved for treatment of multidrug-resistant tuberculosis, Indian Journal of Pharmacology,2013; Vol 45(5): 536-537.
8. https://www.healthline.com/health/pulmonary-tuberculosis
9. Sharma. S.K & Mohan. A, Extrapulmonary tuberculosis, Indian journal of Medical Research, 2004; Vol. 120: 316-353.
10. Edward D Chan, Current medical treatment for tuberculosis, British Medical Journal, 2002; 325(7375): 1282–1286.
11. Haileyesus Getahun, Delphine Sculier, Charalambos Sismanidis, Malgorzata Grzemska, and Mario Raviglione, Prevention, Diagnosis, and Treatment of Tuberculosis in Children and Mothers: Evidence for Action for Maternal, Neonatal, and Child Health Services, Journal of Infectious Disease, 2012; 205(2): S216-S227.
12. https://www.tbfacts.org/tb/

13. Sharma. S.K. & Mohan . A, Multidrug-resistant tuberculosis, Indian J Med Res 120, 2004; 354-376.
14. Anthony S. Fauci, The NIAID Tuberculosis Working Group, Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis: The National Institute of Allergy and Infectious Diseases Research Agenda and Recommendations for Priority Research, 2008; Vol. 197(11): 1493–1498.
15. Ali Akbar Velayati, Parissa Farnia, and Mohammad Reza Masjedi, International Journal of Clinical and Experimental Medicine, 2013; 6(4): 307–309.
16. Todar's online textbook of bacteriology, available at http://textbookofbacteriology.net/tuberculosis.html.
17. Delphi Chatterjee, The mycobacterial cell wall: structure, biosynthesis and sites of drug action, Current Opinion in Chemical Biology 1997; 1: 579-568.
18. P.J. Brennan, Structure, function, and biogenesis of the cell wall of Mycobacterium tuberculosis, Elsevier Science Ltd., 2003; 83, 91–97.
19. Riccardo Manganelli, Roberta Proveddi, Sebastien Rodrigue, Jocelyn Beaucher, Luc Gaudreau, and Issar Smith, σ Factors and Global Gene Regulation in Mycobacterium tuberculosis, Journal of Bacteriology, 2004; 186(4): 895–902.
20. http://www.Genome structure of M.Tuberculosis.org.
21. Gupta. K B, Atreja. A, Transmission of tuberculous infection and its control in health care facilities, National Tuberculosis Institute Bulletin, 2006; 42/3&4, 63 – 67.
22. Parish T, Stoker N, "Mycobacteria: bugs and bugbears (two steps forward and one step back)", Molecular Biotechnology 13 (3) 1999; 191–200.
23. http://en.wikipedia.org/wiki/Mycobacterium tuberculosis.
24. Anishetty S, Pulimi M, Pennathur G: Potential drug targets in Mycobacterium tuberculosis through metabolic pathway analysis. Comput Biol Chem. 2005, 29 (5): 368-378.
25. Hasan S, Daugelat S, Rao PS, Schreiber M: Prioritizing genomic drug targets in pathogens: application to Mycobacterium tuberculosis. PLoS Comput Biol. 2006.
26. Gunter Harth and Marcus A. Horwitz Inhibition of Mycobacterium tuberculosis Glutamine Synthetase as a Novel Antibiotic Strategy against Tuberculosis: Demonstration of Efficiancy In-vivo infect Immun. 2003; 71(1): 456-464.

27. Gunter Harth, Daniel L. Clemens, and Marcus A. Horwitz, Glutamine synthetase of Mycobacterium tuberculosis: Extracellula release and characterization of its enzymatic activity, Proceedings of the National Academy of Sciences of the United States of America, 1994; Vol. 91: 9342-9346.
28. https://www.rcsb.org/3d-view/3ZXR/1
29. Nicolaou CA, Brown N, "Multi-objective optimization methods in drug design". Drug Discovery Today. Technologies, 2013; 10 (3): 427–35.
30. Douglas B. Kitchen, Helene Decornez, John R. Furr and Jürgen Bajorath, Docking and Scoring in Virtual Screening for Drug Discovery: Methods and Applications, Nature Reviews Drug Discovery, 2004; 3(11): 935-949.
31. Anil Koul, Eric Arnoult, Nacer Lounis, Jerome Guillemont & Koen Andries, The challenge of new drug discovery for Tuberculosis, Nature International journal of science, 2011;Vol. 469, 483–490.
32. Soma Mandal, Mee'nal Moudgil, Sanat K. Mandal, Rational drug design, European Journal of Pharmacology, 2009; Vol. 625: 90–100.
33. Sehgal VK, Das S, Vardhan A, Computer aided drug designing, The International Journal of Medical and Dental Sciences, 2017; Vol. 6(1): 1433-1437.
34. Tropsha A, "QSAR in Drug Discovery". In Reynolds CH, Merz KM, Ringe D. Drug Design: Structure- and Ligand-Based Approaches, Cambridge, UK: Cambridge University Press, 2010; 151–164.
35. Anderson AC,"The process of structure-based drug design". Chemistry & Biology, 2003; 10 (9): 787–97.
36. Klebe G, "Recent developments in structure-based drug design". Journal of Molecular Medicine, 2000; 78 (5): 269–81.
37. Akbar Ali, Mohamed EI Badawy, Raza Shah, Wajid Rehman, Yeldez El kilany, El Sayed H El Ashry and Nawaz Tahir, Synthesis, Characterization and *In-Silico* ADMET Screening of Mono- and Di-carbmethoxylated 6,6'-Methylenebis(2-cyclohexyl-4-methylphenol) and Their Hydrazides and Hydrazones, Pelagia Research Library, Der Chemica Sinica, 2017; 8(4): 446-460.

38. Gunter Harth and Marcus A. Horwitz, An Inhibitor of Exported Mycobacterium tuberculosis Glutamine Synthetase Selectively Blocks the Growth of Pathogenic Mycobacteria in Axenic Culture and in Human Monocytes: Extracellular Proteins as Potential Novel Drug Targets, Journal of Experimental Medicine, 1999; 189(9): 1425–1436.
39. Michael V. Tullius, Günter Harth and Marcus A. Horwitz, Glutamine Synthetase GlnA1 Is Essential for Growth of Mycobacterium tuberculosis in Human THP-1 Macrophages and Guinea Pigs, Journal of Clinical Microbiology, 2003; Vol. 71(7): 3927-3936.
40. Lukasz Berlicki, Inhibitors of Glutamine Synthetase and their Potential Application in Medicine, Mini-Reviews in Medicinal Chemistry, 2008; Vol. 8(9): 869 – 878.
41. O Lagerlund, Design and Synthesis of novel Glutamine Synthetase Inhibitors and Development of Palladium(0) - Catalyzed Aminocarbonylation, Acta Universitatis Upsaliensis, 2009; 95: 55.
42. Wojciech W. Krajewski, T. Alwyn Jones, and Sherry L. Mowbray, Structure of Mycobacterium tuberculosis glutamine synthetase in complex with a transition-state mimic provides functional insights, Proceedings of the National Academy of Sciences of the United States of America, 2005; 102(30): 10499–10504.
43. Keerthi Kumar C. T, Keshavayya J, Rajesh.T, Peethambar S. K And Shoukat Ali R. A, Synthesis, Characterization and Antimicrobial Activity of Heterocyclic Azodyes Derived from Thiadiazole, Chemical Science Transactions, 2013, 2(4), 1346-1351.
44. Jitendra Sainy, Ganesh P. Mishra, Rajesh Sharma, Subhash C. Chaturvedi. Synthesis of novel 2-amino 5-Sulfanyl- 1, 3, 4-thiadiazole for Anti Inflammatory and Analgesic agents. Pharmaceutical Chemistry Journal. 2009; 43(1):19-24.
45. Liu Pattan SR, Kittur BS, Sastry BS, Thakur DK., Synthesis of some novel 2-amino 5- substituted 1, 3, 4-thiadiazole for Anti Fungal agents. Indian J. Chem. 2011; 50B, 615-618.
46. Priyabrata Pattanayak. Rajesh Sharma, Prasanta Kumar Sahoo. Synthesis and Evaluation of 2-amino 5-Sulfanyl-1, 3, 4-thiadiazole as Antidepressant, Anxiolytic, and Anticonvuslant agents. Medicinal Chemistry Research Journal, 2009; (18): 351-361.

47. Stephen Turner, Malcolm Myers, Brian Gadie, Timothy L. Synthesis of Antihypertensive of 1, 3, 4-thiadiazole of some 2-aryl 5-guanidino derivatives. Journal of Medicinal Chemistry, 31(5): 906-13.
48. Francesca Clerici, Donato Pocar, Maddalena Guido, Antonella Loche, and Vincenzo Perlini Mario Brufani, Synthesis of 2-Amino-5-sulfanyl-1,3,4-thiadiazole Derivatives and Evaluation of Their Antidepressant and Anxiolytic Activity, Journal of Medicinal Chemistry, 2001; 44 (6): 931–936.
49. BijoMathew, JeradSuresh, Anbazhagan.S and Chidambaranathan.N, Discovery of some novel imines of 2-amino, 5-thio, 1,3,4-thiadiazole as mucomembranous protector. Synthesis, anti-oxidant activity and *in silico* PASS approach, Journal of Saudi Chemical Society, 2016; Vol. 20(1): S426-S432.
50. Foroumadi A, Asadipour A, Mirzaeim, Karimi J, Emamai S. Synthesis Evaluation of invitro anti tuberculosis activity of some 2-(5-nitro-2-furyl)-1, 3, 4-thiadiazole derivatives. PubMed. 2002; 57(9): 765-769.
51. Noolvi MN, Patel HM, Navdeep Singh S, Gadad AK . Synthesis and anti tubercular evaluation of imidazo [2,1-b] [1,3,4] thiadiazole derivatives. Arabian Journal of Chemistry. 2013; 54(5): 183-190.
52. Gadad AK, Noolvi MN, Karpoormath RV. Synthesis and antitubercular activity of a series of 2-sulfonamido/trifluoromethyl-6-substituted imidazo [2, 1-b]-1, 3, 4-thiadiazole derivatives. Bio org Med Chem. 2004; 12(4): 5651-5659.
53. Shiradkar M, Kale R. Condensed bridgehead nitrogen heterocyclic systems: microwave assisted synthesis and bioactivity of s-triazolo[3,4-b][1,3,4]thiadiazoles, s-triazolo [3,4- b][1,3,4]thiadiazines and s-triazolo [3’,4’:2,3]-thiadiazino[5,6-b] quinoxaline. Indian Journal of Chemistry. 2005; 45(1): 1009-1013.
54. Palkar MB, Noolvi MN, Maddi VS, Ghatale M, Nargund LG. Synthesis, spectral studies and biological evaluation of novel series of 2-substituted-5,6-diarylsubstituted imidazo[2,1-b]-1,3,4-thiadiazole derivatives as possible anti tubercular agents. Medicinal Chemistry Research Journal. 2012; 21(1): 1313-1321.
55. Zi-long Tang, Zan-Wen Xia, Zhao-Xu Wang. Synthesis and fungicidal Activity of Novel 2-Aryl-3-[1, 3, 4-thiadiazolyl-6[8]-Methyl-1, 3, 4 benzoxazines. Bioorganic and Medicinal Chemistry.2015; 25[16]: 3378-3381.

56. Sandeep Miglani, Monika Mishra, Pooja Chawla. The rapid Synthesis of Schiff-bases without Solvent under microwave irradiation and their antimicrobial activity. Der Pharma Chemica. 2012: 4(6):2265-2269.
57. Camille G.Wermuth, Similarity in drugs: reflections on analogue design, Drug Discovery Today, 2006; Vol. 11(7–8): 348-354.
58. Pranita P. Kore, Madhavi M. Mutha, Rishikesh V. Antre, Rajesh J. Oswal, and Sandip S. Kshirsagar, Computer-Aided Drug Design: An Innovative Tool for Modeling, Open Journal of Medicinal Chemistry, 2012; 2: 139-148.
59. Frederick W.Goldberg, Jason G.Kettle, ThierryKogej, Matthew W.D.Perry and Nick P.Tomkinson, Designing novel building blocks is an overlooked strategy to improve compound quality, 2015; Vol. 20(1): 11-17.
60. Brian Leonard, Jorge Coronel, Mark Siedner, Louis Grandjean, Luz Caviedes, Pilar Navarro, Robert H. Gilman and David A. J. Moore, Inter- and Intra-Assay Reproducibility of Microplate Alamar Blue Assay Results for Isoniazid, Rifampicin, Ethambutol, Streptomycin, Ciprofloxacin, and Capreomycin Drug Susceptibility Testing of *Mycobacterium tuberculosis*, Journal of Clinical Microbiology, 2008; Vol. 46(10): 3526-3529.
61. Vanitha JD, Paramasivan CN. Evaluation of Micro plate Alamar blue assay for drug susceptibility testing of Mycobacterium avium complex isolates**.** Diagnostic Microbiology and Infectious Disease, 2004; 49: 179-182.
62. Franzblau SG, Witzig RS, McLaughlin JC, Torres P, Madico G, Hernandez A, Degnan M T, Cook MB, Quenzer VK, Ferguson RM, Gilman RH. Rapid lower technology MIC determination with clinical Mycobacterium tuberculosis isolates by using the Micro plate Alamar blue assay. Journal of Clinical Microbiology: 1998; 36: 362-366.
63. Jose D Jesus Alba-Romero et.al, Application of the Alamar Blue Assay to Determine the Susceptibility to Anti-tuberculosis Pharmaceuticals. African Research Journals of Microbiology. 2011; 5 (26):4659-4666.
64. Erik Walum, Acute Oral Toxicity, Environmental Health Perspectives, 1998; Vol. 106(2: 497-503.

65. Gerald L. Kennedy, Jr,t Rayanne L. Ferenz and Bruce A. Burgess, Estimation of Acute Oral Toxicity in Rats by Determination of the Approximate Lethal Dose Rather Than the LD_{50}, 1986; Vol. 6(3): 145-148.
66. https://www.definitions.net/definition/does/U.S. National Library of Medicine
67. Matthew P. Baumgartner and Carlos J. Camacho, Choosing the Optimal Rigid Receptor for Docking and Scoring in the CSAR 2013/2014 Experiment, Journal of Chemical Information and Modelling, 2016; 56 (6): 1004–1012.
68. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (March 2001). Experimental And computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv.Drug Deliv.2001;P.3-26.
69. Lipinski CA, Lead-and drug-like compounds: the rule - of-five revolutions. Drug Discovery Today: Technologies, 2004; 1(4): 337- 341.
70. Nilufer Solak and Sevim Rollas, Synthesis and antituberculosis activity of 2-(aryl/alkylamino)-5-(4-aminophenyl)-1,3,4-thiadiazoles and their Schiff bases, ARKIVOC Journal, 2006; (xii): 173-181.
71. Himaja Malipeddi, Asif A Karigar, Venkata Ramana Malipeddi and Mukesh S Sikarwar, Synthesis and Antitubercular Activity of Some Novel Thiazolidinone Derivatives, Tropical Journal of Pharmaceutical Research ,2012; 11 (4): 611-620.
72. Kai Zhang, Peng Wang, Li-Na Xuan, Xiao-Yun Fu, Fen Jing, Sha Li, Yu-Ming Liu, and Bao-Quan Chen, Synthesis and antitumor activities of novel hybrid molecules containing 1,3,4-oxadiazole and 1,3,4-thiadiazole bearing Schiff base moiety, Bioorganic and Medicinal Chemistry Letters, 2014; http://dx.doi.org/10.1016/j.bmcl.2014.09.086
73. https://chem.libretexts.org/Textbook_Maps/Organic_Chemistry_Textbook_Maps/Map%3A_Organic_Chemistry_with_a_Biological_Emphasis_(Soderberg)/11%3A_Nucleophilic_carbonyl_addition_reactions/11.6%3A_Imine_(Schiff_base)_formation.
74. Cho S, Lee H S, Franzblau S, Microplate Alamar Blue Assay (MABA) and Low Oxygen Recovery Assay (LORA) for Mycobacterium tuberculosis, Mycobacteria Protocols, Methods in Molecular Biology, 2015; Vol. 1285: 281-292.

75. Ekwall. B, Silano. V, Paganuzzi Stammati. A and Zucco. F, Toxicity Tests with Mammalian Cell Cultures, Short-term Toxicity Tests for Non-genotoxic Effects, 1990; 75-97.
76. David J. Andrew, Acute Toixicity, General, Applied and Systems Toxicology, Wiley Online Library, 2009.
77. Vichai, V. and Kirtikara, K., 'Sulforhodamine B colorimetric assay for cytotoxicity screening',2006; 1(3):1112–1116.
78. Sharma Y.R, Organic Spectroscopy, Fourth and revised multicolour edition, S Chand and company, 2012.
79. Sharma Y.R, Textbook of Elemental Organic Spectroscopy.
80. Kalsi P.S, Spectroscopy of Organic Compounds, sixth edition, New age International Pvt. Ltd.,
81. Gerdeep R Chatwal, Sham K Anand, Instrumental methods of chemical analysis, Himalayan Publishing House, 2005; fifth edition.

ANNEXURE

Proceedings of the Chairperson, Institutional Animal Ethics Committee, Madras Medical College, Chennai – 3.

Present: Dr.Sudha Seshayyan, M.B.B.S, M.S (Anatomy)

Roc. No. 17/ AEL/IAEC/MMC Date: 15.03.2018.

Sub: Animal Experimental laboratory – IAEC – research Project – approval – regarding.

Ref: IAEC meeting held on 06.09.2017.

The following order is based on the meeting held on 06.09.2017 and the addendum issued on 15.03.2018.

Project ID.	1 7/17.
CPCSEA registration number	1917 / ReBi/S/16/CPCSEA /25.10.2016
Name of the Researcher	Ayyamperumal.E M. Pharm II year, Department of Pharmaceutical Chemistry
Name of the Guide	Dr.A.Jerad Suresh,M.Pharm, Ph.D.,
Title of the project	Design, Synthesis, Characterization and Biological Evaluation of some Novel Thiadiazole (schiff's base) Derivatives as Anti-Tubercular Agents against Glutamine synthetase I
Date of submission of proposal to IAEC	01.08.2017
Date on which IAEC conducted	06.09.2017
Date of submission of modified proposal (if applicable)	14.03.2018
Date on which approved	15.03.2018
Validity of the approved proposal	1 year
Remarks	Albino mice – Female 24 numbers approved.

Chairperson
Institutional Animal Ethics Committee
Madras Medical College
Chennai -3

To,
Dr.A.Jerad Suresh,M.Pharm,Ph.D.,
Prof. & Head of Dept of Pharmaceutical Chemistry,
College of Pharmacy,
MMC, Chennai -3.

Copy to
Special Veterinary Officer, Animal Experimental Laboratory
Madras Medical College, Chennai – 3.

"Skill and Will to Make and Serve Quality Pill"

69th IPC 2017

CHANDIGARH

22nd - 24th December, 2017

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Certificate

This is to certify that

Prof./Dr./Mr./Ms. AYYAM PERUMAL E

has participated as Delegate / Volunteer

in the 69th Indian Pharmaceutical Congress

held at Chitkara University, Rajpura from December 22nd to 24th, 2017.

Dr. Mahesh Burande	Dr. Shailendra Saraf	Dr. Dhirender Kaushik	Dr. Ashish Baldi
President - IPCA	Chairman - LOC	Organizing Secretary	Chairman, Registration Committee - LOC

Organised by : Indian Pharmaceutical Congress Association (IPCA)

Hosted by : Association of Pharmaceutical Teachers of India (APTI)

68th INDIAN PHARMACEUTICAL CONGRESS

ipc 2016 VISAKHAPATNAM

Theme Quality Pharmaceuticals and Patient Welfare

PHARMA VISION 2020 — swasth bharat — PHARMACISTS FOR A HEALTHY INDIA

Certificate of Participation

This is to certify that Prof./Dr./Mr./Ms. AYYAMPERUMAL E

of .....

has attended the 68th IPC as Registered Delegate held at AU College of Pharmaceutical Sciences,

Andhra University, Visakhapatnam, A.P. during 16th – 18th December 2016.

Mr. S.V. Veerramani	Dr. Rao Vadlamudi	Dr. T.V. Narayana	Dr. G. Nagarjuna Reddy
President, IPCA-2016	LOC, Chairman	LOC, Secretary	Chairman, Reg. Com.

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SRM University, Kattankulathur - 603 203.

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-has attended one day workshop on "High Performance Thin Layer Chromatography (HPTLC) *and its Applications"*

organized by Interdisciplinary Institute of Indian System of Medicine (IIISM), *SRM University, Kattankulathur- 603 203.*

on 10th September 2016.

Dr. K. Ilango
Convener
Dean - IIISM

Prof. D. Narayana Rao
Director – Research

Dr. K. S. Jayachandran
ANCHROM Enterprises (I) Pvt. Ltd.
Mumbai.

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FACULTY OF PHARMACY
55th NATIONAL PHARMACY WEEK CELEBRATIONS

Certificate

This is to certify that Mr./Ms. E. AYYAMPERUMAL had participated as a delegate in the workshop on Infra-Red Spectroscopy / Gas Chromatography held on 22nd November 2016 organized by Faculty of Pharmacy, Sri Ramachandra University, Porur, Chennai.

This workshop carries 2 credit hours

D. Chamundeeswari
Dr. D. Chamundeeswari
Principal

Dr. K. V. Somasundaram
Dean of Faculties

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE, CHENNAI - 03.

WORKSHOP ON DRUG DESIGN

Certificate

This is to certify that Mr. / ~~Ms~~/ ~~Mrs~~. E. AYYAMPERUMAL *of....* COLLEGE OF PHARMACY.,.. MMC *.....has attended the WORKSHOP ON DRUG DESIGN AND HANDS ON TRAINING on 24th & 25th of June and July 9th - 2016 at the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai - 600 003.*

Dr.A.Jerad Suresh
Principal
College of Pharmacy, MMC.

Dr.N.Jayashree
Co-ordinator
College of Pharmacy, MMC.

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SRM University

SRM Nagar, Kattankulathur - 603 203.

ONE DAY WORKSHOP ON

ATOMIC ABSORPTION SPECTROSCOPY (AAS) & ITS APPLICATIONS

Certificate

This is to Certify that Mr. /~~Ms./Dr.~~ Ayyamperumal E *has attended one day workshop on "Atomic Absorption Spectroscopy (AAS) & Its Application" organized by Interdisciplinary Institute of Indian System of Medicine (IIISM), SRM University, Kattankulathur- 603 203. on 19th November 2016.*

Prof. K. Ilango
Convener
Dean - IIISM

Prof. D. Narayana Rao
Director – Research
SRM University

K. Philip Sudhagar
Branch Manager
Labindia Analytical Instruments Pvt. Ltd.,
Mumbai

NATIONAL SEMINAR ON "RECENT ADVANCES IN HIV / AIDS DRUG DISCOVERY AND DEVELOPMENT"

December 27 & 28, 2016

Certificate of Participation

This to certify that Prof. / ~~Dr.~~ / Mr. / ~~Mrs.~~ / ~~Ms.~~ E. AYYAM PERUMAL

has actively participated ~~as a Delegate/ Chaired a Session/ Delivered guest lecture/ Presented a paper (oral/poster)~~

~~entitled~~ .....

.....

in National Seminar on "Recent Advances In HIV / AIDS Drug Discovery and Development" held at Kalasalingam University, Krishnankoil from 27th - 28th, December 2016.

Organized by

Kalasalingam University, Krishnankoil - 626126 in association with

Antiviral Research Society - Regn. No. 144/2016 (TN Soc. Regn. Act)

Dr. P. Selvam
President, AVRS

Dr. S. Saravana Sankar
Vice Chancellor

COLLEGE OF PHARMACY

MADRAS MEDICAL COLLEGE

CHENNAI - 600 003.

Certificate

This is to certify that Mr. / ~~Ms.~~ AYYAMPERUMAL . E ,

a student of II M.PHARM , *College of Pharmacy, Madras Medical College, Chennai has attended the* ***"Lecture series-2018"*** *conducted by the College of Pharmacy, Madras Medical College, Chennai - 03 on 11.01.2018.*

Dr. N. Jayshree, M.Pharm, Ph.D.,
Organizing Secretary,
Professor of Pharmacology,
Madras Medical College, Chennai.

Dr. A. Jerad Suresh, M.Pharm, Ph.D., M.B.A.,
Principal,
College of Pharmacy,
Madras Medical College, Chennai.